Escola Superior
Agrária
Politécnico de Coimbra

REQUERIMENTO DE ENTREGA DEFINITIVA
Tese de Dissertação / Trabalho de Projeto / Relatório de Estágio

André Reis Pereira, estudante com o nº 20140169, do Curso de Mestrado em Agricultura biológica na área de especialização em Recursos genéticos, venho por este meio entregar a versão definitiva da Tese de Dissertação, em formato digital, de acordo com o disposto no Regulamento do IPC aplicável ao 2º Ciclo.

Título da Publicação: EVALUATION AND CHARACTERIZATION OF PORTUGUESE MAIZE LANDRACES. ENHANCING GENETIC DIVERSITY

Orientador(es): Pedro Mendes-Moreira

Ano de conclusão: __2022____

Para os devidos efeitos, declaro que autorizo o depósito da versão digital definitiva supramencionada no Repositório Institucional do IPC, integrado no Repositório Científico de Acesso Aberto de Portugal (RCAAP) e de Recolha de Informação de Registo Nacional de Teses e Dissertações (RENATES), nos termos da legislação em vigor.

Declaro, ainda, que o depósito da versão definitiva da Tese de Dissertação no Repositório Institucional, se encontra sujeito às condições abaixo indicadas (assinalar a opção pretendida):

- ☐ Livre Acesso.
- ☐ Acesso Embargado por:
  - ☐ 6 meses
  - ☐ 1 Ano
  - ☒ __2 anos__________

Justificação (no caso de embargo): A tese vai ser utilizada para publicação científica______________________________

O/A Autor(a):

Data: __25_ / _02__ / ___2022____

____
(Assinatura)

Assinado por: ANDRÉ REIS PEREIRA
Identificação: [illegible]
Data: 2022-02-25 às [illegible]

Escola Superior Agrária
Politécnico de Coimbra

Master in Organic Farming

Professional internship report

EVALUATION AND CHARACTERIZATION OF PORTUGUESE MAIZE LANDRACES. ENHANCING GENETIC DIVERSITY

André Reis Pereira

Coimbra, 2022

Escola Superior
Agrária
Politécnico de Coimbra

Master in Organic Farming

Professional internship report

EVALUATION AND CHARACTERIZATION OF PORTUGUESE MAIZE LANDRACES. ENHANCING GENETIC DIVERSITY

Internal Advisor: Pedro Mendes Moreira, PhD

Internship: IPC-ESAC

André Reis Pereira

Coimbra, 2022

To my mother, she always believed in me, even when she didn't.

Acknowledgments:

Professor Pedro Mendes Moreira, for his direction, support, encouragement, clarification of doubts, and assistance when I needed it. Thank you also for all of the knowledge shared, as well as all of the comments and criticisms that contributed to my learning and improvement.

Ana Lusa Neri, my Research Fellow in the LIVESEED H2020 project, for his encouragement, willingness, and friendship over both years of trials.

Duarte Pintado, my Research Fellow in the LIVESEED H2020 project, for his encouragement and assistance at the start of my long internship.

LIVESEED H2020 project and its entire organization, as well as everyone who directly or indirectly assisted me in completing this work.

Sousa Valley farmers; Sr Antonio Alves and Luis Tiago (and families) for their help and full access to their land.

Ader-Sousa (Eng. Claudia) for all of her assistance during the testing and farmer meetings.

Dr. Manuela Veloso for supplying the seeds that allowed this research to be carried out.

My family, especially my brothers (3) and mother, for their encouragement, support, and motivation.

Abstract

Genetic resources and Organic farming can be naturally linked by Participatory Plant Breeding (PPB) programs. Together they represent a powerful tool to tackle the unavailability of seed, breed in organic environments faced by European organic farmers. Escola Superior Agrária de Coimbra (ESAC-IPC) continues the "VASO" mission (Participatory Breeding Program, begun in 1984) in collaboration with farmers and stakeholders from the Sousa Valley. Over the last decade, breeding efforts have been directed toward organic farming and low-input agriculture systems, where the diversity, distinctiveness, and adaptability of Portuguese maize germplasm may be fully exploited.

The study is divided into two trials. Both aim to phenotypically characterize, test the agronomic behavior and adaptation of the Portuguese maize populations for two years (2020-2021), providing a subsequent identification of the most suitable populations for each environment.

The Organic vs Conventional trial was performed in two locations distance 1.3 km and intended to compare the maize landraces behavior in two different agro-systems low input organic agriculture versus conventional agriculture (50 accessions).

The On-farm PPB trial was conducted in two locations in Sousa Valley with the goal of comparing the behavior of maize landraces in different farm locations (agroecological zone). Furthermore, was possible to evaluate the maize populations throughout germplasm evaluations done by farmers and stakeholders, to provide future breeding goals (10 accessions).

Registered data included days-to-anthesis, days-to-silk, Grain yield (Kg/ha), Phenotypic evaluations using HUNTERS descriptor (Plant Height, Uniformity, Leaf aNgle, Tassel, Ear placement, Root and Stalk lodging) from 2020 and 2021, and the biometric parameters associated with the ear from 2020.

Maize populations that were more fitted to organic environments and agroecological systems were identified using ANOVA, PCA, and Simple Rank analysis (Sousa Valley). On-field assessments and Plant Participatory breeding initiatives helped to improve the farmer and stakeholder network.

Keywords: *Zea mays*; Genetic Resources; Maize Landraces; HUNTERS descriptor; Participatory Plant Breeding, Organic Farming.

Resumo

Os recursos genéticos e a agricultura biológica podem ser vinculados por programas de Melhoramento Participativo (PPB). Juntos, eles representam uma ferramenta poderosa para enfrentar a indisponibilidade de sementes produzidas em agricultura biológica, que enfrentam os agricultores europeus em modo de produção biológica.

A Escola Superior Agrária de Coimbra (ESAC-IPC) continua a missão “VASO” (Programa de Melhoramento Participativo que começou em 1984) junto dos agricultores e stakeholders do Vale do Sousa. Nos últimos 10 anos, os objetivos de melhoramento foram orientados para a agricultura biológica e sistemas agrícolas de baixo insumo, onde a diversidade, singularidade e adaptação dos recursos genéticos de milho português podem ser verdadeiramente aproveitadas.

O estudo é dividido em dois ensaios. Ambos visam: caracterizar fenotipicamente, testar o comportamento agronómico e a adaptação das populações portuguesas de milho durante dois anos (2020-2021) proporcionando uma posterior identificação das populações mais indicadas para cada ambiente.

O ensaio Orgânico vs. Convencional foi realizado em dois locais a 1,3 km de distância e teve como objetivo comparar o comportamento das variedades tradicionais portuguesas de milho em dois sistemas agrícolas com filosofia oposta; agricultura biológica versus agricultura convencional (50 acessos testados).

O ensaio On-farm PPB foi realizado em duas localidades no Vale do Sousa com o objetivo de comparar o comportamento de variedades tradicionais portuguesas de milho em diferentes explorações agrícolas (zona agro-ecológica) no Vale do Sousa. Para alem disso, realizou-se, através de uma perspetiva multi-actor, avaliações de germoplasma com agricultores e stakeholders, de forma a selecionar as variedades tradicionais e os objetivos de melhoramento mais adequados para cada sistema (10 acessos testados).

Os dados avaliados de 2020 e 2021 incluem: dias para antese, dias para libertação das sedas, produção estimada em grão (Kg/ha), avaliações fenotípicas usando o descritor HUNTERS (altura da planta, uniformidade, ângulo da folha, bandeira, colocação da espiga, acama e plantas partidas) e os parâmetros biométricos associados à espiga de 2020.

As análises: ANOVA, PCA e Simple Rank permitiram identificar as populações de milho mais adequadas a ambientes de produção biológica e a sistemas agroecológicos (Vale do Sousa). As avaliações em campo e os esforços de melhoramento participativo reforçaram a rede de agricultores e stakeholders envolvidos.

Palavras-chave: *Zea mays*; Recursos genéticos; Variedades Locais de Milho; HUNTERS; Caracterização; Melhoramento Participativo de Plantas, Agricultura Biológica.

Contents

List of Figures

List of Tables

Chapter 1- Introduction

The maize god Hun Hunahpu affirms the Mayan society's (750 a.C.) unique and deeper connection with this crucial crop, which enabled for their progress and livelihood (The British Museum, 2020). Today, it is hardly less significant as the foundation of numerous meals and one of the world's three most important cereal crops, alongside wheat and rice. Maize is grown on 193.7 million hectares worldwide (ICAR, 2021).

Figure 1 shows the world maize production by country in 2018/19 (Shahbanden, 2019). The United States was the main producer with 366.3 million Megagrams (Mg). China and Brazil finish the top 3 maize-producing countries (Shahbanden, 2019).

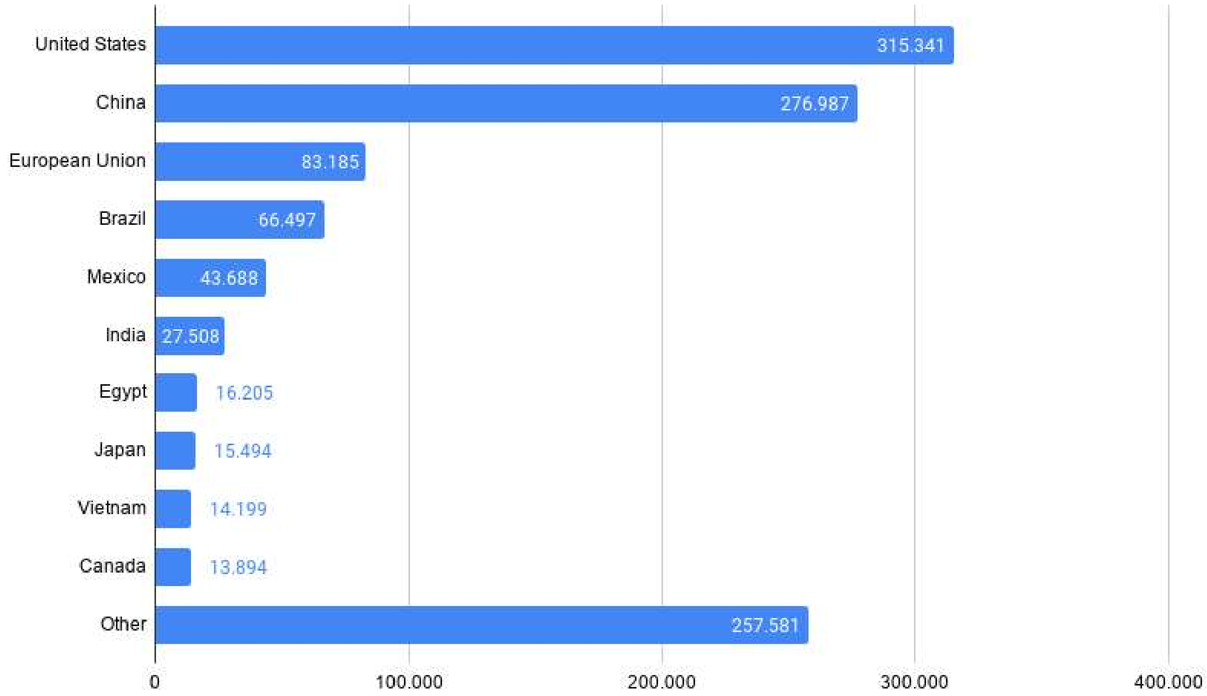


Figure 1- Global maize production in 2018/2019, by country (Shahbanden, 2019)

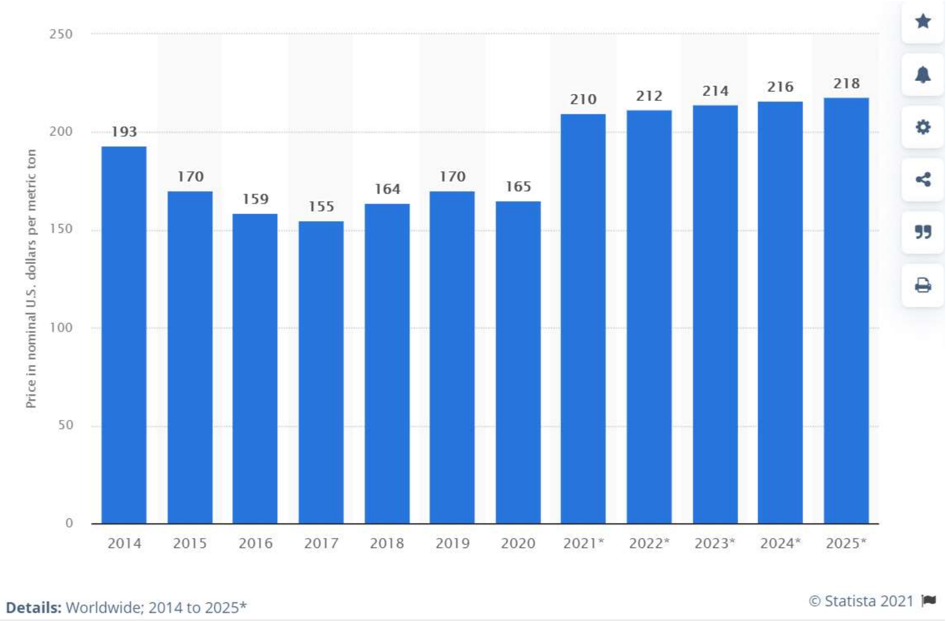
Maize's economic significance is shown by the various types of its utilization, which range from animal and human food to the high-tech fuel industry (Carena, 2013; Karaman et al., 2021). Maize is used raw or processed in around 4000 products (Karaman et al., 2021).

The maize sector in Portugal is experiencing one of its most significant crises as a result of low producer prices and growing production costs (Anpromis, 2020; Serrano & Ges, 2016). According to ANPROMIS (2020), maize (grain) sown area has gradually reduced in recent years (2013-2020). Despite this, maize remains the most significant arable crop in the Portuguese agricultural setting, with the largest sown area (66 390 hectares) (Anpromis,2020; INE, 2020). In 2020, total maize (grain) production in Portugal was 681 939 Mg, a 9.7 percent decrease from 2019 and a 5.4 percent decrease from the five-year average (INE, 2020).

The rising need for food in emerging countries has had a significant impact on maize demand, which is expected to outstrip demand for wheat and rice (Njagi, 2019; Stevens

& Madani, 2016). A good example is Ethiopia, where maize represents 65% of total food calories consumed by households (Njagi, 2019).

Climate change impacts on maize output are predicted to have a significant impact on food security (Guna et al., 2019; Stevens & Madani, 2016). As a result, as indicated in Figure 2, an increase in prices is projected during the following five years. The pressing need to address both food and environmental challenges highlights the need of sustainable agricultural practices such as organic farming (Fuss et al., 2018).



Details: Worldwide; 2014 to 2025*

© Statista 2021

Figure 2- Average prices for maize worldwide from 2014 to 2025 (Plecher, 2021).

In Europe, there is a significant dedication to organic farming; plans such as The Green Deal Farm to Fork aim to have 25% of agricultural European land under organic cultivation by 2030 (European Commission, 2021).

The European organic regulation EC 848/2018 stated that derogations for all plant reproductive material would be prohibited by the end of 2035 (Frederic Rey et al., 2021; Fuss et al., 2018). As a result of this new and sustainable legislative framework, the maize industry will be impacted, resulting in an urgent need to adapt cultivars to organic farming and other sustainable agricultural systems (Solfanelli et al., 2019).

Maize genetic resources (Landraces, Populations) in Portugal are commonly connected with maize bread, an old, fundamental, and traditional meal of the Mediterranean diet (Alves et al., 2018; Frederic Rey et al., 2021; Revilla et al., 2022; Vaz Patto et al., 2008). These neglected genetic resources may provide organic farmers with a broader choice of options, can be grown under organic farming settings through participatory maize breeding programs, and can be suited to the farmer as well as the market (Mendes-Moreira et al., 2014).

Exploration and evaluation of genetic resources is critical not just for conservation but also for supplying a more diversified variety of foods. However, these pre-breeding efforts and their relation to organic farming are still rather restricted; thus, during trials, it is vital to modify and encourage the use of local genetic resources with organic farmers (LIVESEED, 2020; McLean-Rodríguez et al., 2019).

In the following sections of this chapter, the literature background is covered.

1.2- Plant Genetic Resources conservation

Plant Genetic Resources are considered an invaluable heritage of humanity and their loss is an irreversible process, mainly involving global food security (Bioversity International, 2019; Ebert & Engels, 2020; Hossain et al., 2019). Since the late 1960s, the conservation of genetic resources has been a major concern. Some irreparable losses arise primarily as a result of disuse, representing the loss of millennia of domestication, selection, and adaption to edaphoclimatic conditions (Bioversity International, 2020; Peres, 2016).

The International Biological Programme (IBP) and FAO (the United Nations Food and Agriculture Organization) joined forces to form the International Plant Genetic Resources Institute (IPGRI), now known as Bioversity International, to combat genetic degradation (Bioversity International, 2020; IPGRI, 2000).

The three main strategies utilized in plant genetic resources conservation are conservation *ex-situ*, conservation *in-situ,* and *on-farm* conservation (Ebert & Engels, 2020; Ortiz et al., 2010).

Ex-situ conservation entails the preservation of viable populations of species or representative parts of biodiversity that have scientific, economic, and social value outside of their natural habitat (GenBank). The major goal of this sort of conservation is to maintain and preserve genetic heterogeneity and variation (Andjelkovic & Polje, 2016; Ebert & Engels, 2020; Ortiz et al., 2010).

Conservation *in situ* – Management of genetic resources in their natural settings or, in the case of domesticated and cultivated species, in conditions where their distinct qualities can emerge. This sort of conservation helps organisms to continue their evolution. This management encourages changes in the genetic pattern in favor of wildlife adaption, preservation, and maintenance (Cuena-Lombraña et al., 2020; Ortiz et al., 2010).

On-farm conservation can be thought of as a complementary technique to in-situ conservation since it allows species to continue their evolutionary process and demonstrates the importance of communities in preserving and promoting genetic variation (Mendes-Moreira et al., 2014; Vaz Patto et al., 2008).

On-Farm conservation (static) focuses on the diversity of one specific landrace held inside the targeted agriculture system, whereas *On-Farm* management (dynamic) focuses on increasing landrace diversity in any on-farm system (ECPGR, 2017).

According to ECPGR, 2017, Farmers' cultivation of landraces, underutilized cultivars, and heterogeneous populations can be viewed as both on-farm conservation and on-farm management, delivering one or more of the following benefits at various scales:

1. Complementary conservation approach linked to *ex-situ* collections
2. Conservation and development of cultural landscapes
3. Conservation and development of crop diversity originating in Europe and its linked traditions
4. Conservation and development of diversity that is not covered by the formal sector
5. Mitigation of genetic erosion
6. Crop evolution and adaptation to changing conditions in the field
7. Diversification of agriculture and consequently increased consumer choice, ecosystem benefits, and services

8. Opportunities for sustaining current, and developing potential, niche markets.

1.2- Plant Genetic Resources and Organic Agriculture

Organic agriculture differs from conventional agriculture in that it employs a broader spectrum of diversity (LIVESEED, 2020). Crop rotations are valued in organic farming, and the use of a diverse range of crops can be advantageously integrated with the use of locally adapted cultivars or landraces (Kovács & Pedersen, 2019).

Nowadays, cultivars for organic agriculture are obtained in three ways: i) cultivars from conventional breeding (derogations); ii) cultivars bred in conventional environments for organic agriculture; and iii) organic cultivars, in which the entire breeding process is carried out under organic conditions and in accordance with organic principles (Frederic Rey et al., 2021). Because organic seed supply in Europe is currently insufficient for many crops, farmers can obtain exemptions (derogations) to use conventional seed (untreated after harvest) instead (Solfanelli et al., 2019). However, the new EU organic regulation (2018/848/EU) dates the expiration of derogations until the end of 2035, increasing the urgency to promote a larger range of cultivars to organic producers.

Within this context, European projects such as LIVESEED have arisen to increase the transparency and competitiveness of the organic seed and breeding business, hence encouraging wider usage of organic seed (LIVESEED, 2020). LIVESEED main goals are:

- Foster harmonized implementation of the EU organic regulation on organic seed
- Strengthen organic seed databases in the whole EU
- Investigate socio-economic aspects related to production and use of organic seed
- Improve availability and quality of organic seed
- Develop guidelines for organic cultivar testing and registration
- Develop innovative breeding approaches suited to organic farming
- Widen the choice of organic cultivars meeting the demand of farmers, processors, retailers, and consumers
- Research activities of LIVESEED will cover five main crop categories (legumes, vegetables, fruit trees, cereals, and fodder crops) considering different farming systems and pedoclimatic zones across Europe (LIVESEED, 2020).

Pre-breeding activities such as cultivar testing can be used to connect genetic resources to organic breeding projects (Nass & Paterniani, 2000; Ortiz et al., 2010). Identifying and transmitting adaptability and resistance traits found in wild parents and landraces to prospective cultivars (Nass & Paterniani, 2000; S. Sharma & Carena, 2016). The chosen germplasm is crossed and transferred to a chosen intermediary group of germplasm that is predicted to be useful in an organic breeding program (Figure 3). This method has the potential to increase genetic variability and broaden its genetic range (S. Sharma & Carena, 2016).

Furthermore, focusing on cultivar evaluation and plant breeding for organic farming can help to close the yield gap between conventional and organic systems (Crespo-Herrera & Ortiz, 2015).

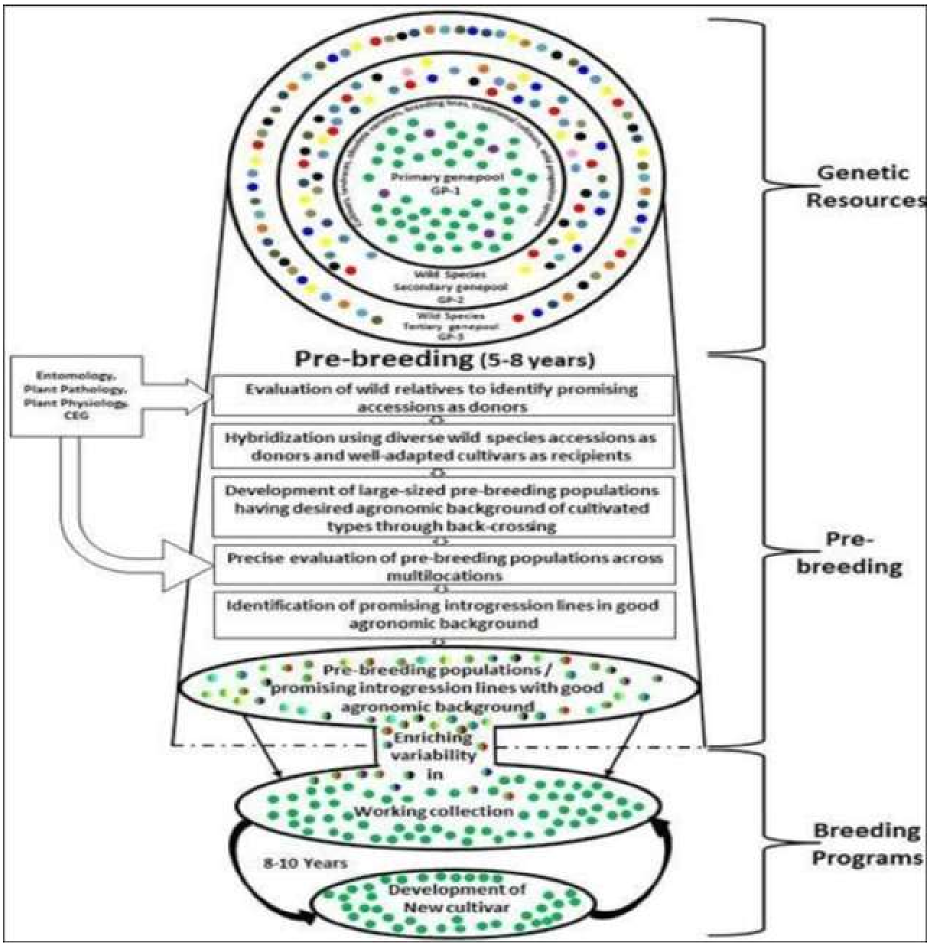


Figure 3-Pre-breeding as a bridge between genetic resources and crop breeding (Sharma, 2017).

Crop evaluation and subsequent characterization is an important activity in the utilization of wild relatives or landraces, and it consists of data collecting to define, identify, and distinguish accessions of the same crop species (IPGRI, 2000; Mendes-Moreira, 2015; Ortiz et al., 2010). This is based on observations (qualitative variables) or measurements (quantitative variables) of a variety of morphological characteristics (Bioversity International, 2007; Gotor et al., 2008; S. Sharma & Carena, 2016; Sharma, 2017).

Effective morphological characterization should allow for relatively simple differentiation of phenotypes and provide the first values of variability (diversity) within the gene pool (Andorf et al., 2019; Burle & Oliveira, 2010). This characterisation is highly important for identifying germplasm and providing a measure of the genetic integrity of the conserved accessions (Vernooy et al., 2020). It is capable of identifying existing accesses in collections, distinguishing accesses with common traits, and detecting duplicate resources (Burle & Oliveira, 2010).

Finally, uniformity of descriptors is an important part of morphological characterisation. Because one of the goals is to facilitate the interchange and use of germplasm, it is advised that the morphological descriptors be as uniform as feasible (Vernooy et al., 2020). It is critical to use descriptors issued by international organizations and to apply them exactly as published so that the information is understood by users worldwide (Andorf et al., 2019; Cancellier et al., 2011; Vernooy et al., 2020).

1.3- Maize origin and domestication

Maize resulted from multiple independent domestications from its wild progenitor, teosinte (*Z. mays* ssp. *parviglumis*) (Doebley et al., 2006; Matsuoka et al., 2002). The cradle or original location of maize domestication is said to be the Balsas River region in México, where teosinte still grows endemically (Andjelkovic et al., 2017; Ortiz et al., 2010; Serratos Hernández, 2009).

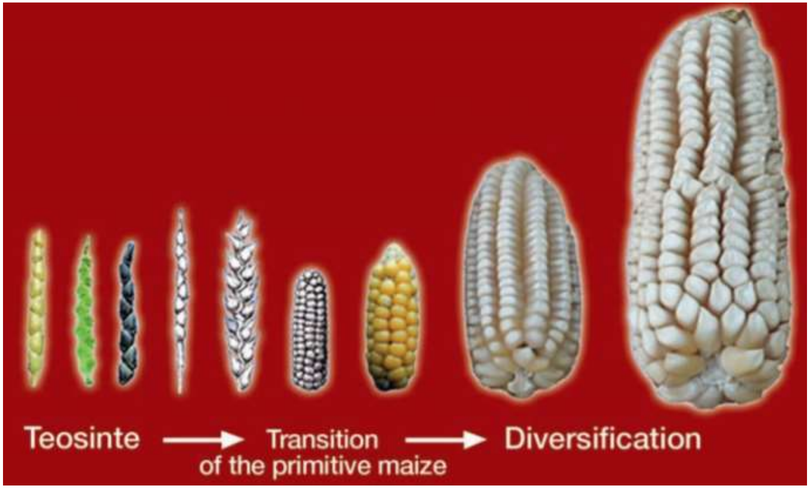


Figure 4-Morphological sequence of the possible evolution of the ear from teosinte to maize. ***Elaborated by Antonio Serratos*** (Serratos Hernández, 2009)

Maize was introduced into Europe shortly after Columbus' arrival in America, around the end of the 15th century, in Southern Spain. It was initially grown in gardens for its beauty and exoticism before its food value revealed its significance (Hallauer, 2007; Love, 2021). This crop spread over the world, being produced on a commercial scale between the latitudes of 58° North (Russia) and 40° South (South Africa) (Argentina) (Leff et al., 2004).

Maize was brought to Portugal in the Mondego fields in the 16th century and quickly expanded throughout the North, Center, South, Azores, and Madeira archipelagos (Ferrão, 1992; Vaz Patto et al., 2008). During the 17th and 18th centuries, maize is established and expanded. Its application to polyculture (Maize-Beans) transformed food availability in the Portuguese rural communities (Vaz Patto et al., 2008).

Hallauer, (1994) indicated that maize domestication and breeding can be divided into 4 distinct phases:

The first phase is characterized by the evolution from the wild state (teosinte) to become a domesticated species, which is a basis for adaptations to the latitude, altitude, and precipitation levels.

In the second phase, 250 varieties of maize, with middling distinct traits, were selected by the Native American civilizations to meet the food demand of animals and human beings.

In the third phase the development of distinct varieties (Maize Landraces) from the germplasm coming from the previous phase, by the Native Americans and the Europeans after its introduction (1500–1925). This development and selection of traits in the varieties are carried out to obtain different types of grain, colors, textures, cycles, and uses.

The fourth phase started with the development of pure line and hybrid concepts (1909 to the present) and indicate the beginning of hybrid maize breeding.

The third and fourth phases took place in Portugal. The third phase began in the 16th century, when maize began to expand, resulting in the selection of several landraces that were phenotypically distinct, adapted to different edaphoclimatic features and farmers' demands (Mendes-Moreira et al., 2014; Vaz Patto et al., 2008).

The fourth stage began after World War II, when Portugal introduced American hybrids that, after being suited to Portuguese conditions, replaced landraces (Mendes-Moreira et al., 2009; Vaz Patto et al., 2008).

1.4- Maize landraces

Maize landraces are dynamic populations with distinct traits and geographic historical origin, they are genetically diverse, locally adapted, and associated with farmers' breeding purposes (McLean-Rodríguez et al., 2019; Vaz Patto et al., 2008). A variety of environmental and bio-cultural factors influence their phenotypic and genetic diversity, resulting in various unique Open-pollinated Varieties (OPV) (Kutka, 2011; McLean-Rodríguez et al., 2019).

Since maize domestication and development of landraces seed production was usually done throughout the phenotypic mass selection, it relies on the relative heritability of the traits and the influence of environmental effects cannot be separated (Wolff, 1972; Mendes-Moreira et al., 2009). However, the procedure was straightforward; it only required one generation every cycle and allowed for the screening of a huge number of plants while conserving genetic variety and preventing inbreeding (Kutka, 2011; WOLFF, 1972).

OPVs are less productive than commercial hybrid cultivars (Kutka, 2011). However, they represent a strong source of genetic variability due to the high adaptation potential for specific environmental conditions (Perales H. R., Brush S. B., 2003; Vaz Patto et al., 2008). They can be investigated in the quest for genes that are tolerant and/or resistant to biotic and abiotic stimuli, as well as for commercially significant-quality features (Araújo & Nass, 2002; Moreira, 2006).

It is difficult, competitive, and expensive to search for superior features in genotypes such as yielding ability, disease, insect resistance, stress tolerance, or improved nutritional quality (Nass & Paterniani, 2000). Breeders tend to focus on adapted and enhanced materials, avoiding wild parents and landraces because they would take a long time, a lot of money, and it would be difficult to find potentially beneficial genes (Araújo & Nass, 2002; Kutka, 2011).

More than 95% of the Portuguese maize landraces are flint white grain, selected for the production of maize bread (Alves et al., 2019; Revilla et al., 2022; Vaz Patto et al., 2008). When compared to commercial cultivars, are related with high protein content and low viscosity levels, according to Alves et al., 2019. However, this diversity of germplasm was never thoroughly explored in order to meet stringent quality demand (Vaz Patto et al., 2008).

1.5- Hybrid breeding

Maize's appropriate reproductive organization, with separate male and female flowers on the same plant, benefits greatly from heterosis, which was discovered at the beginning of the twentieth century when continuous self-pollination caused a significant reduction in vigor and yield levels, which can be improved through cross-pollination (Andorf et al., 2019; Crow, 1998). Inbred lines can capture the desirable traits and produce homozygous plants enhancing the efficiency of hybrid crosses. Although, it requires continuous self-pollination of a selected maize variety or population over a period of five to seven generations (Andorf et al., 2019; Carena, 2013).

This concept of inbred lines and hybrids allowed for human control over self-pollination for inbred line development and cross-pollination for hybrid production (Crow, 1998; Kutka, 2011). The yield boost observed in the 1940s in the United States (Figure 5) was mostly attributable to the widespread adoption of the inbreed-hybrid technique. Nonetheless, the increased use of fertilizer, increased stand, and herbicide use were all major factors (Crow, 1998).

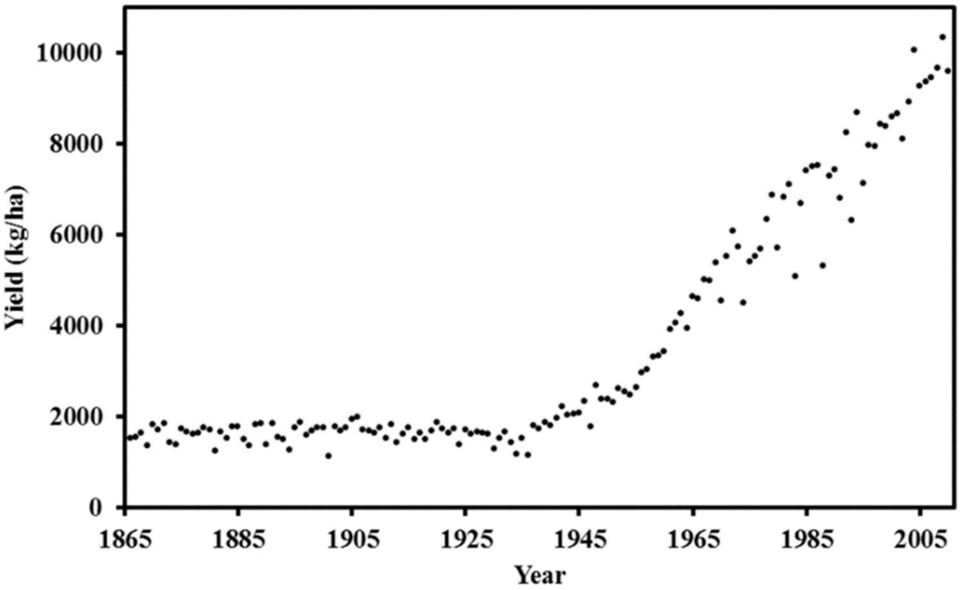


Figure 5- Average USA maize yields 1866–2010. Data are available from USDA.

Now our understanding of heterosis in maize is based and informed by genomics (Andorf et al., 2019). Plant breeders and geneticists were able to study and map locations on the chromosomes to identify useful genes and their quantitative traits loci (QTLs) narrowing and sharpening the breeding process which meant a reduction of cost and time (Andorf et al., 2019).

Inbred lines developed by several generations of self-pollination have been largely replaced by doubled haploid (DH) lines which require only two generations to develop (Andorf et al., 2019; Josia et al., 2021).

Genome selection followed by genome editing will be the future of maize breeding (Andorf et al., 2019). While certain American countries legally embrace these technologies, several European countries are disputing their usage because there is still no legal clarity in the use of GMOs (Genetically Modified Organisms) (Andorf et al., 2019; Menz et al., 2020).

GMOs can have a harmful influence on the environment not only because they require the use of pesticides and artificial fertilizers. Their possible negative environmental implications include genes that can be passed to native species and result in herbicide-resistant weeds, a loss in crop biodiversity due to their frequent usage, and detrimental affects on bird populations, insects, and soil biota (FAO, 2003; Husaini & Sohail, 2018). These potential environmental consequences are incompatible with the organic agriculture principles as established by the International Federation of Organic Agricultural Movements (IFOAM) (IFOAM, 2021).

1.6 - Participatory Plant Breeding

Participatory plant breeding (PPB) entails active collaboration between researchers, farmers, and other actors throughout the breeding process, making it more effective to reach and support small farmers' preferences and needs, which are often overlooked in the standardized conventional breeding chain (S Ceccarelli, 2012; Colley et al., 2021).

According to Ceccarelli (2019), participatory breeding efforts on 47 crops have been applied in 69 nations (10 developed and 59 developing) over a 36-year period, with a greater influence in developing countries (Salvatore Ceccarelli & Grando, 2019). A review published in 2021 identified 47 PPB projects across the United States, Canada, and Europe that include 22 crop species (Colley et al., 2021). It demonstrates that PPB can adapt to a wide range of contexts and aims, and its evolution is causing significant changes in the way plant genetic resources are handled, valorized, and implemented in sustainable agriculture systems such as organic agriculture (Colley et al., 2021).

PPB emerges as a modern and suitable response to the growing demand for novel cultivars adapted to organic farming, as it provides more effective and low-cost management of production means by employing farmers' fields for breeding and seed production (Mendes-Moreira et al., 2009, 2014; Vaz Patto et al., 2008). Breeders and farmers collaborate through a multi-actor approach (Mendes-Moreira et al., 2017; Serpolay et al., 2018), this relationship relies on the opportunity to make decisions during the various stages of the process, allowing the farmer to participate and define breeding objectives and priorities (Mendes-Moreira et al., 2014; Serpolay et al., 2018).

The primary goals of the PPB are to boost both output and profit by developing and adapting suitable cultivars and, as a result, developing breeder skills in farmers themselves to carry on the sustainable production of seeds (Mendes-Moreira et al., 2017). From a maize breeding perspective, Participatory Plant Breeding (PPB) can be included in the third phase of maize domestication, with methodologies of the fourth phase applied (Hallauer; A., 1994).

There are only three maize PPB projects in the Global North, one in the US, one in Portugal, and a third project that has recently been initiated in France (Colley et al., 2021).

According to Mendes-Moreira (2006), PPB in Portugal began in 1984 in Vale do Sousa (directed by Dr. Silas Pêgo). This project was carried out by NUMI (Ncleo de Melhoramento do Milho Português), which became aware of the genetic erosion occurring in Portugal with maize genetic resources and decided to conduct maize germplasm collection missions in Portugal with the assistance of FAO. The efforts of conservation and administration of genetic material acquired aided in the development of both the VASO project and the first Portuguese germplasm bank (BPGV).

Although *ex-situ* conservation allowed for material retention, it did not allow for germplasm coevolution in response to environmental changes or possible crop enemies. Furthermore, there is a gap between what the breeder needs to know about the agronomic behavior of the germplasm and what the curator can supply. Dr. Silas Pêgo developed the HUNTERS descriptor (High, Uniformity, aNgle, Tassel, Ear, Root lodging, and Stalk lodging) within this framework to perform a summary and expeditious characterization of the biometric parameters of the plant and the ear, providing the information needed to adapt and valorize the Portuguese germplasm to suitable environments and suitable farmers (Alves et al., 2018; Mendes-Moreira et al., 2009, 2018).

According to Mendes-Moreira, 2018, throughout history the objectives of VASO have included:

- Participatory breeding of traditional, composite, and synthetic maize varieties for human consumption,
- Development of selection methodologies in farmers,
- Improvement of agricultural systems regarding sustainability,
- Improving the knowledge about maize genetics, food technology, and food quality.
- Intensify the links between actors in the bread seed chain network,
- Portuguese maize genetic resources characterization and agronomic evaluation *on-farm.*

In contrast to the productivist philosophy, VASO concentrates on the so-called integrant philosophy (Moreira, 2006). This model's main focus is the farmer, who is the system's most essential resource and from whom decisions are made (Moreira, 2006).

In recent years, VASO's research has been geared toward organic agricultural methods and other low-input systems, in response to the exponential rise of organic agriculture in Portugal in recent decades (INE, 2020; Mendes-Moreira et al., 2018).

1.7- Aims

The main objective of this work is to valorize Portuguese maize genetic resources, display their productive and phenological traits, and promote their use in organic farming and sustainable agriculture systems. Within this scope, two trials with different objectives were performed in 2020 and repeated in 2021.

The first trial (Organic Vs Conventional) aims to evaluate and compare de agronomic behavior of 50 Portuguese maize populations in two opposed agriculture systems, the low-input Organic system (Caldeirão) versus the Conventional system (Vagem). Grain yield evaluations and Grain yield rank will allow the selection of the best genotypes to integrate into Organic Participatory Plant Breeding programs (PPB). Morphological characterization was performed using the HUNTERS descriptor to display their (maize populations) phenological traits and further on correlate all the phenotypic data with Grain yield performance.

The second trial (On-Farm PPB) aims to evaluate and compare the agronomic behavior of 10 Portuguese maize populations in two locations in the Sousa Valley region (Macieira de Lixa and Lousada - agroecological sites[1]), under farmers' practices and conditions in a low-input Conventional system. Grain yield evaluations and Grain yield rank will allow the selection of the best genotypes to integrate into Participatory Plant Breeding programs ongoing in Sousa Valley in these two locations. Morphological characterization was performed using the HUNTERS descriptor to display their (maize populations) phenological traits and further on correlate all the phenotypic data with Grain yield performance. From the tested 10 maize populations, 6 were chosen; 3 genotypes of 'Verdeal' (VA), and 3 of 'Pigarro' (Pg) that represent different years of mass selection in different environments and gastronomic potential for maize bread. These 6 populations were tested in two years in three environments (Macieira de Lixa, Lousada, and the Organic Caldeirão in Coimbra). This framework allows the identification of maize populations with organic gastronomic potential (Maize Bread) and the best selection environments suitable to use in PPB programs adapted to Organic and low-input farming. Finally, the On-Farm PPB study aspired to be a showcase for Sousa Valley farmers and stakeholders, who were requested to evaluate five major morphological features and maize aptitude (gastronomic or animal feed) of the maize populations evaluated at the Macieira de Lixa trial site (15th of October of 2020). Attempting to combine farmer and market perspectives on selection and breeding aims.

---

[1] Polyculture and other agroecological approaches, family farming.

Chapter 2- Organic vs Conventional trial

2.1- Trial Locations

The Organic vs Conventional trial was managed at the low input organic field and conventional field of IPC-ESAC in Bencanta, Coimbra, Portugal (40.21709426119619; -8.44779968261719; 15 m elevation). The two places distance 1,3 km (Figure 6).

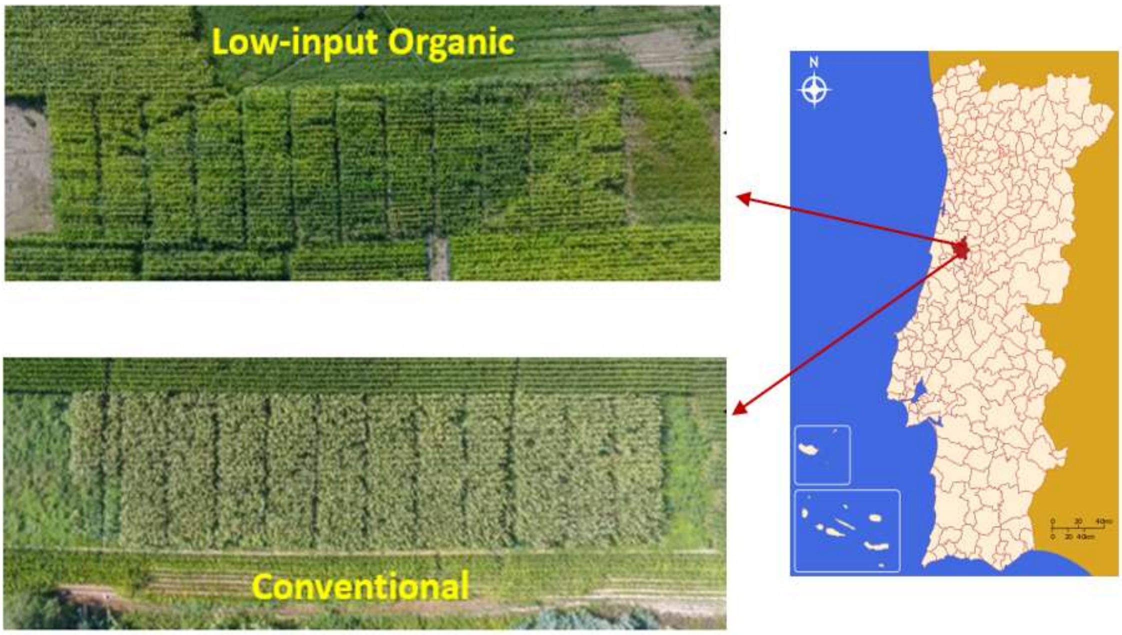


Figure 6- Overview of the trial sites in low-input organic (Caldeirão), and Conventional (Vagem). (Drone photos by filipe hanower (2020), map photo from wikipédia)

2.2- Trial edaphoclimatic conditions

The climate of Coimbra is hot and temperate, with more rain in the winter. According to Koppen and Geiser's classification, Coimbra's climate is Csa, which is a Mediterranean climate with hot summers (Climate, 2021; Kottek et al., 2006). According to the data collected by the meteorological station of ESAC that refer to 2020 (**Annex I**), the area shows an average annual temperature of 16,5°C and an average month temperature of 19,2°C in May, 19,6°C in June, 23,7°C in July, 21,7°C in August and 20,9°C at September (ESAC, 2020).

Soil samples were obtained after trial implantation to measure physical and nutritional soil properties; the results are shown in Table 1.

Table 1- Soil analysis of the trial sites in 2020 and 2021 (Annex II-IV)

Organic Vs Conventional trial					
Parameter	**Unit**	**Organic Caldeirão 2020**	**Conventional Vagem 2020**	**Organic Caldeirão 2021**	**Conventional Vagem 2021**
Soil texture		Medium	Medium	Medium	Medium
Soil <2mm	**%**	78,5	79,04	79,12	88,74
Org. Mat	**%**	1,9 (low)	1,6 (low)	1,9 (low)	2,2 (medium)
pH	**H2O**	7,5 (neutral)	5,9 (few acid)	7,2 (neutral)	5,8 (few acid)
P_2O_5	**mg/kg**	265 (very high)	319 (very high)	330 (very high)	78 (medium)
k_2O	**mg/kg**	318 (very high)	265 (very high)	356 (very high)	370 (very high)

2.3- Germplasm used

The germplasm used in the 2020 and 2021 trials (Table 2) included:

- 41 open-pollinated populations (OPPs) from Azores (Portugal), used by the farmers for food (e.g. maize bread and polenta) and feed; 36 OPPs were collected in a collection mission on both Terceira and S. Miguel Islands (Figure 7 and 8), between 1979 and 1982 (Bettencourt and Gusmão, 1982), and kept in cold storage at Estação Agronómica Nacional and then moved to Banco Português de Germoplasma Vegetal (BPGV) at Braga (Portugal) and multiplied in 2018 and 2019 in the low-input organic field, at Escola Superior Agrária de Coimbra (ESAC); the remaining 5 Azorean OPPs were donated by farmers and collected by Duarte Pintado e Emanuel Ferreira in 2018 - BSM17, BT17, BT18, VT17, MT17.

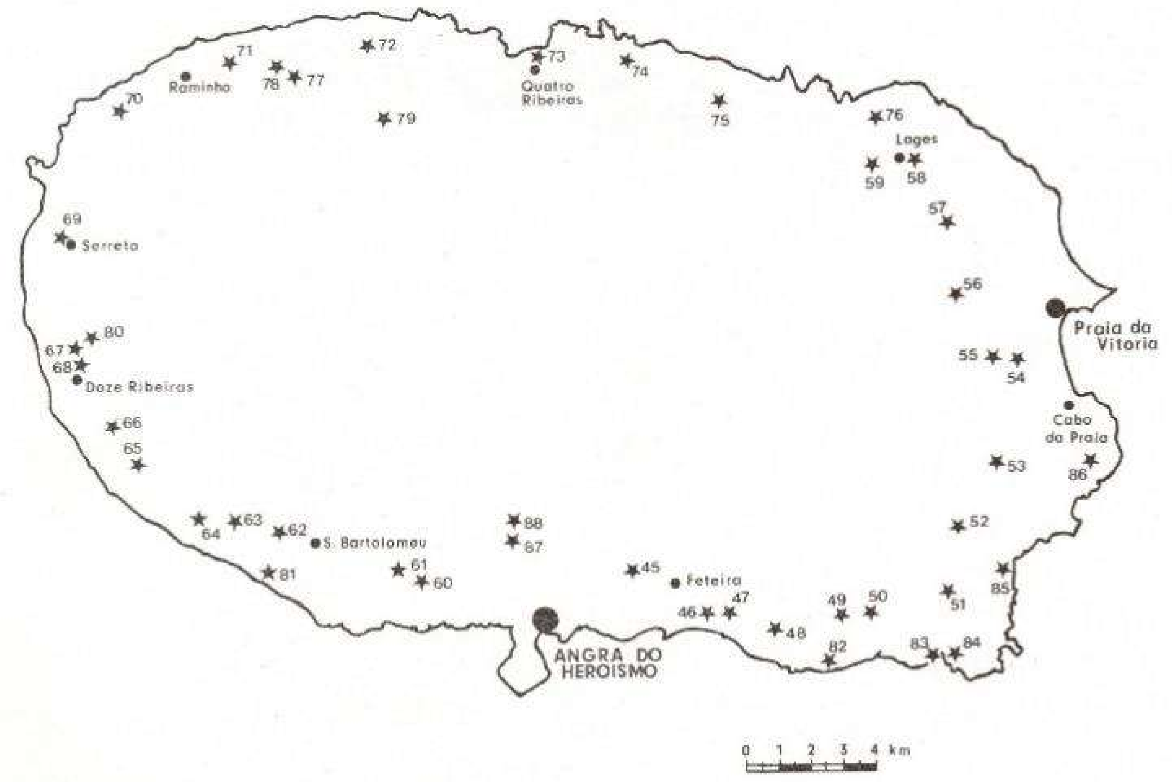


Figure 7- Places where collections were carried out on Terceira Island. The mission took place in 1979.

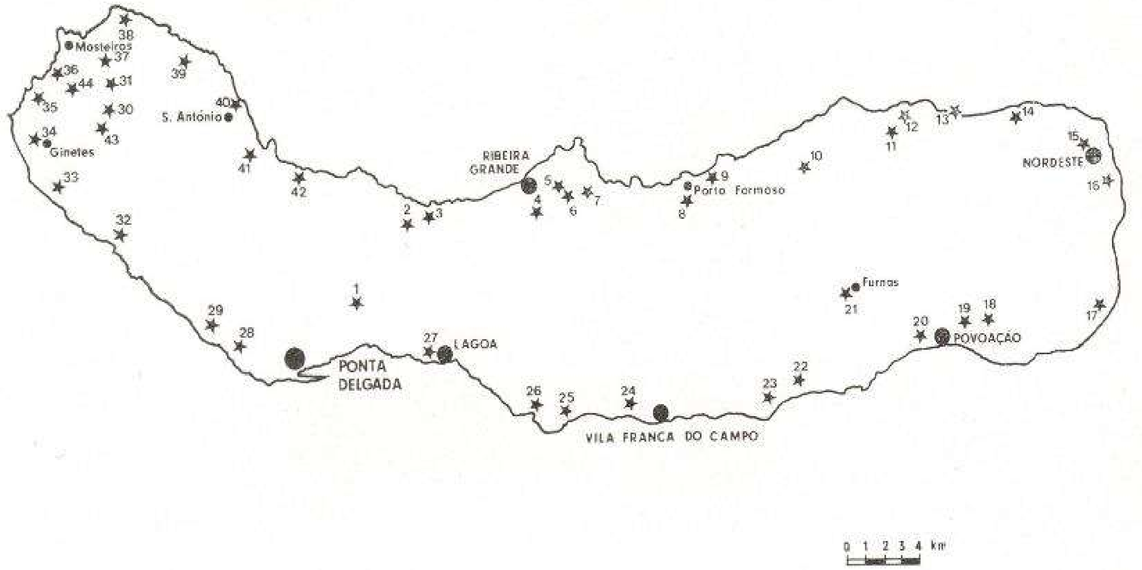


Figure 8-Places where collections were carried out on S. Miguel Island. The mission took place in 1979.

-6 accessions were derived from 2 Open-pollinated populations used by the farmers for food (Maize bread), obtained from the participatory plant breeding program "VASO" in Sousa Valley that has been active in the northern part of Portugal since 1984:

- ‘Pigarro’ is a white flint type FAO 300 cycle, with high kernel-row numbers (18 to 28), selected from a traditional Portuguese landrace (3 accessions: Pg 19 (Lou 19); Pg 18 (Cald 18) and Pg 19 (Cald 19) - subjected to stratified mass selection in ESAC in 2018 and 2019).
- ‘Verdeal´ is a white flint type FAO 300 cycle, selected from a traditional Portuguese landrace (3 accessions: VA 17 (Sequeiro Lousada 2017), VA 19 (Reg Lou 19), VA 19 (Sequeiro Lou 19) - subjected to stratified mass selection on-farm in Lousada, Portugal in different irrigation systems in 2017 and 2019).

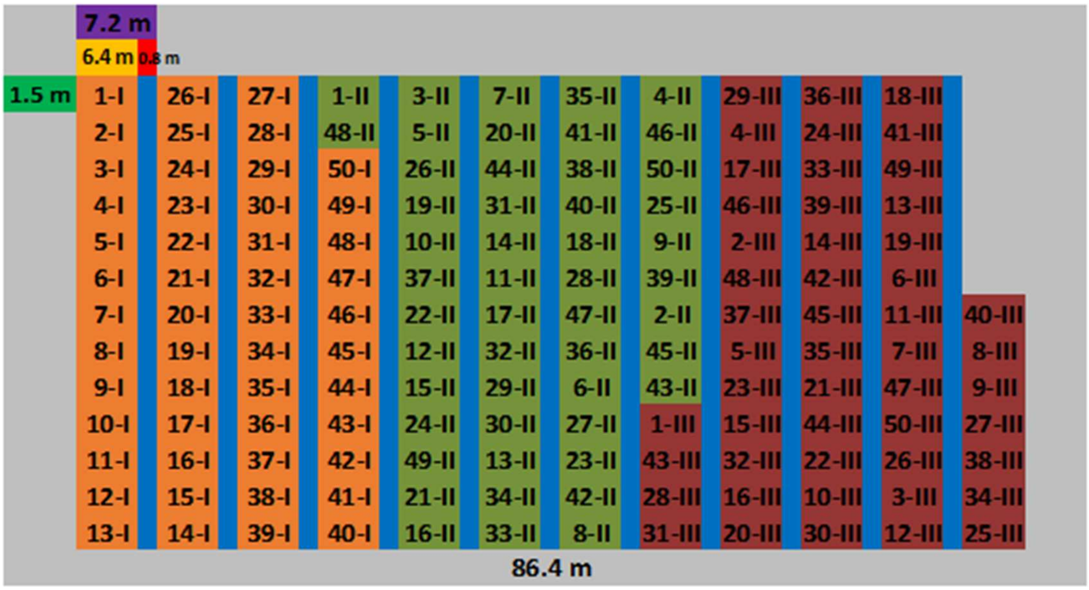
-3 Composite Cross Populations (CCP) obtained using a polycross method based on 'Nutica' experience (Mendes-Moreira et al., 2009): 'SinPre' (Sintético Precoce) obtained through the crossing of 12 maize populations (10 Portuguese landraces and 2 American populations) subjected to on-farm stratified mass selection under PPB ("VASO") and multiplied in 2019 at ESAC. ‘BulkAzores1’ and ‘BulkAzores2’ were obtained in 2018-19 through the crossing of 40 Azorean OPPs and subjected to mass selection at ESAC and on-farm in 2019.

Table 2- The germplasm used in 2020 and 2021 in the Organic Vs Conventional trial.

Organic Vs Conventional Trial (germplasm used)			
Genotype	**Origin**	**Genotype**	**Origin**
2444	Azores, Portugal	**2522**	Azores, Portugal
2448	Azores, Portugal	**2524**	Azores, Portugal
2449	Azores, Portugal	**2525**	Azores, Portugal
2488	Azores, Portugal	**2526**	Azores, Portugal
2489	Azores, Portugal	**2527**	Azores, Portugal
2493	Azores, Portugal	**2528**	Azores, Portugal
2494	Azores, Portugal	**2529**	Azores, Portugal
2496	Azores, Portugal	**2530**	Azores, Portugal
2498	Azores, Portugal	**2531**	Azores, Portugal
2499	Azores, Portugal	**VT17 - (Cald 18)**	Azores, Portugal
2501	Azores, Portugal	**MT17 - (Cald 18)**	Azores, Portugal
2502	Azores, Portugal	**MONJ-3-(Cald 18)**	Azores, Portugal
2504	Azores, Portugal	**MONJ-2 (Cald 18)**	Azores, Portugal
2505	Azores, Portugal	**BT18 - (Cald 19)**	Azores, Portugal
2507	Azores, Portugal	**BT17 - (Cald 18)**	Azores, Portugal
2508	Azores, Portugal	**BSM17- (Cald 18)**	Azores, Portugal
2509	Azores, Portugal	**Bulk-Azores1 - CCP**	Coimbra, Portugal
2510	Azores, Portugal	**Pg 18**	Vale do Sousa Portugal
2513	Azores, Portugal	**Pg 19**	Vale do Sousa Portugal
2514	Azores, Portugal	**Pg 19 - (Lous 19)**	Vale do Sousa Portugal
2515	Azores, Portugal	**SinPre - CCP**	Vale do Sousa Portugal
2516	Azores, Portugal	**VA 17 - (Seq Lou 17)**	Vale do Sousa Portugal
2517	Azores, Portugal	**VA 19 - (Reg Lou 19)**	Vale do Sousa Portugal
2518	Azores, Portugal	**VA 19 - (Seq Lou 17)**	Vale do Sousa Portugal
2519	Azores, Portugal	**Bulk-Azores 2 - CCP**	Coimbra, Portugal

2.4- Experimental design

The accessions were evaluated in a randomized complete block (RCBD) design with 60 000 stands, in plots of two lines with 6.4 m lengths and 0,8 m interrow distance (Figure 9).



1-I	26-I	27-I	1-II	3-II	7-II	35-II	4-II	29-III	36-III	18-III	
2-I	25-I	28-I	48-II	5-II	20-II	41-II	46-II	4-III	24-III	41-III	
3-I	24-I	29-I	50-I	26-II	44-II	38-II	50-II	17-III	33-III	49-III	
4-I	23-I	30-I	49-I	19-II	31-II	40-II	25-II	46-III	39-III	13-III	
5-I	22-I	31-I	48-I	10-II	14-II	18-II	9-II	2-III	14-III	19-III	
6-I	21-I	32-I	47-I	37-II	11-II	28-II	39-II	48-III	42-III	6-III	
7-I	20-I	33-I	46-I	22-II	17-II	47-II	2-II	37-III	45-III	11-III	40-III
8-I	19-I	34-I	45-I	12-II	32-II	36-II	45-II	5-III	35-III	7-III	8-III
9-I	18-I	35-I	44-I	15-II	29-II	6-II	43-II	23-III	21-III	47-III	9-III
10-I	17-I	36-I	43-I	24-II	30-II	27-II	1-III	15-III	44-III	50-III	27-III
11-I	16-I	37-I	42-I	49-II	13-II	23-II	43-III	32-III	22-III	26-III	38-III
12-I	15-I	38-I	41-I	21-II	34-II	42-II	28-III	16-III	10-III	3-III	34-III
13-I	14-I	39-I	40-I	16-II	33-II	8-II	31-III	20-III	30-III	12-III	25-III

Figure 9- Organic vs Conventional trial design in 2020 and 2021

2.5- Agronomic practices

In both years soil fertilization was done in the same way in all environments.

- **Organic Caldeirão**- No fertilization
- **Conventional Vagem**- conventional maize hybrid synthetic fertilization (Overall represents: 100 kg/ha of N, 100 kg/ha of P_2O_5, and 200 kg/ha of k_2O).

The field activities in the trial site during the two years (2020-2021) are described in Tables 3 and 4.

Table 3-Field activities during the year 2020

Site	Ploughing	Seedbed Preparation	Design of the sowing Lines	Sowing	Weed Control	Harvest
Cardeirão-ESAC (Organic)	15/05/2020	27/05/2020	28/05/2020	02/06/2020	Manual	10/11/2020
Vagem-ESAC (Conventional)	15/05/2020	28/05/2020	28/05/2020	04/06/2020	Mechanic	03/11/2020

Table 4- Field activities during the year 2021

Site	Ploughing	Seedbed Preparation	Design of the sowing Lines	Sowing	Weed Control	Harvest
Cardeirão-ESAC (Organic)	18/05/2021	05/05/2021	09/06/2021	09/06/2021	Manual	12/11/2021
Vagem-ESAC (Conventional)	18/05/2021	30/05/2021	31/05/2021	31/05/2021	Mechanic	01/11/2021

2.6- Agronomic characterization and evaluation

During the maize crop cycle, the biometric parameters of the plant and the biometric parameters associated with the ear of the 50 maize populations were characterized using the HUNTERS descriptor, with a random sample of 10 plants and 10 ears per maize population. Grain yield was calculated using a 60000 stand and 15% moisture.

Silas Pêgo developed the HUNTERS scale (High, Uniformity, Angle, Tassel, Ear, Root lodging, and Stalk lodging) (Table 5) to carry out a brief and expeditious characterization of the materials under study, and it was used in the characterization of germplasm during the SOLIBAM and DIVERSIFOOD projects (Strategies for Organic and Low-input Integrated Breeding and Management) (DIVERSIFOOD, 2016; SOLIBAN, 2015). These measurements allow for the quick study of a wide range of materials, which is very useful for pre-breeding research.

For height measurements, a metrically marked ruler was used. The List of the traits collected in the trials is displayed in Table 5.

Table 5- Measured characteristics and respective descriptions, according to the HUNTERS descriptor ***(MENDES-MOREIRA ET AL., 2017).***

Maize crop characterization - HUNTERS

Traits	**Codes**	**Scale**	**Type of data available**	**How it has been assessed / Description**
Days-to-silk	Fi	Nº days		From planting until 50% of the plants in the plot begin silk emergence
Days-to-anthesis	Mi	Nº days		From planting until 50% of the plants in the plot start anthesis
Days-to-silk-end	Ff	Nº days		From planting until 50% of the plants in the plot finish silk emergence
Days-to-anthesis-end	Mf	Nº days		From planting until 50% of the plants in the plot finish anthesis
Stand*		Plants ha^{-1}		Thousands of plants per hectare;
Moisture	MO	%	Plot (considering 4 ears)	The grain moisture was measured with the ISOELECTRIC GRAIN CHECK® moisture meter, using the grain of the average four ears;
Overlap Index	OI		Index	This method enables the knowledge of a population concerning the relative amount of theoretical allogamy versus autogamy; $OI = \frac{(Ff - Fi) + (Mf - Mi) - \lvert Ff - Mf \rvert - \lvert Fi - Mi \rvert}{2\,(\mathrm{Ff - Fi})}$
Height	H	cm	20 plants	Plant height, from the stalk basis to the last leaf insertion before the tassel;
Height of the First Ear	H1E	cm	20 plants	Ear height, from the stalk basis to the highest ear bearing node;
Uniformity	U	1 to 9	Plot	1-minimum uniformity and 9 – maximum; 1-4 to pure lines and 5-9 to populations;
Leaf Angle	N	1 to 9	Plot	The angle of the adaxial side of the leaf above the ear with the stalk (5=45°, <5 =<45° and >5 = >45°C);
Tassel Branching	T	1 to 9	Plot	1- absent tassel (Inbreeds and hybrids) 9- a much-branched tassel (frequent in populations with abnormal fasciated ears);

Ear Placement	E	1 to 9	Plot	5- indicates that the ear is located in the middle of the plant, if <5 bellows and if >5 above the middle of the plant;
Root Lodging *	R	%	%	Percentage of plants leaning more than 30º from vertical;
Stalk Lodging *	S	%	%	Percentage of plants broken at or below the primary ear node, related with the quality of the stalk and the stalk damage caused by some insect attack;
Grain Yield		Kg/ha	Plot	Hand harvest, combine used grain yield and moisture content directly measured; Grain yield (60000 stands) 15% moisture=Grain yield/ha x (100% - % moisture at harvest)/(100%-15%moisture)

The biometric parameters associated with the ear (Table 6) examined in this descriptor are those listed in Pêgo & Hallauer (1984) and other parameters developed by Pêgo, for a total of 29 traits (Pêgo, 1982; Pêgo & Hallauer, 1984).

To test grain moisture, an ISOELECTRIC GRAIN CHECK® moisture meter was utilized, and weighing was done using a PLJ 4000-2M KERN® digital scale. A SEED COUNTER - PFEUFFER® grain counter were utilized in the counts, while an ear meter and a micrometer were employed in the measurements.

Table 6- Biometric parameters associated with the ear and their descriptions, according to the HUNTERS descriptor (Mendes-Moreira et al., 2017)

Maize crop characterization - Ear evaluations				
Traits	**Codes**	**Scale**	**Type of data available**	**How it has been assessed / Description**
Ear Length	L	cm	10 ears	Ear length;
Ear Diameter 1 and 3	DE1, DE3	cm	10 ears	Large diameter in the 1/3 bottom and top of the ear respectively (Figure 10);
Ear Diameter 2 and 4	DE2, DE4	cm	10 ears	Small diameter in the 1/3 bottom and top of the ear respectively (90° rotation from large diameter) (Figure 10);
Kernel-Row Number 1 and 2	R1, R2		10 ears	Row number in the 1/3 bottom and top of the ear respectively (nº);
Fasciation	Fa	1 to 9	10 ears	1 – without fasciation and 9 = maximum of fasciation (Figure 11);
Determined vs Indeterminate	D_I		10 ears	Top of the ear full of grain, case of determinate ears (2) or not, case of indeterminate ears (1), the average value is calculated;
Convulsion	CV	0 to 5	10 ears	Kernel row arrangement in the ear (0 - without convulsion, regular kernel row arrangement, 5 – maximum of convulsion, without kernel row arrangement);
Type of grain	F/D		10 ears	1- Popcorn, 2-flint, 3-medium flint, 4-low flint, 5 - 50% flint and 50%dent, 6 - low dent, 7-medium dent, 8-high dent, 9-sweet maize;
Ear Weight	EW15	g	10 ears	Ear weight adjusted to 15% of grain moisture;
Kernel Weight	KW15	g	10 ears	Kernel weight per ear, adjusted to 15% moisture;
Cob Weight/Ear Weight	CW_EW	%	10 ears	Indicates the percentage of cob weight in the ear weight;

Ears Moisture	HR%		10 ears	The grain moisture was measured with the ISOELECTRIC GRAIN CHECK® moisture meter, using the grain per ear and calculating the average;
Kernel Depth	KD	cm	10 ears	The measure of one kernel in the middle of the ear;
Kernel Number	KN		10 ears	Kernel number per ear;
Thousand Kernel Weight	SW15	g	10 ears	Thousand kernels weight at 15% moisture content;
Grain Color	KC		10 ears	reserving the odd numbers for the white group and the even numbers for the yellow group (Table 8).
Kernel per Row	NC		10 ears	Kernel number per row;
Cob Diameter 1 and 3	DC1, DC3	cm	10 ears	Large diameter in the 1/3 bottom and top of the cob respectively;
Cob Diameter 2 and 4	DC2, DC4	cm	10 ears	Small diameter in the 1/3 bottom and top of the cob respectively (90° rotation from large diameter);
Medulla 1 and 2	M1, M2	cm	10 ears	The large and small length of medulla respectively;
Rachis 1 and 2	Ra1, Ra2	cm	10 ears	The large and small length of rachis respectively;
Cob Color	CC		10 ears	Cob color: 1 is red and 2 is white.

Table 7- Evaluation of the type of grain (F/D). (Mendes-Moreira et al., 2017)

Flint grain	**Dent grain**
1 - Popcorn	6 - Weak Dent
2 - Intense Flint	7 - Medium Dent
3 - Medium Flint	8 - Intense Dent
4 – Weak Flint	
5 - Flint /Dent (50%)	9 - Sweet Type

Table 8- Evaluation of grain color. (Mendes-Moreira et al., 2017)

White	**Yellow**
1 - Snow	2 - Lemon
3 - Pearl	4 - Roasted
5 - Drink	6 - Orange
7 - Moreno	8 - Red
	9 - Color segregation

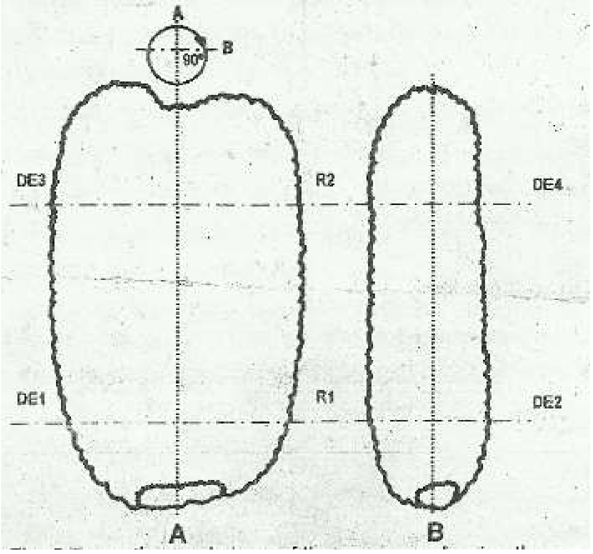


Figure 10- Two orthogonal views of the same ear showing the way the two sets of diameters and the two-line numbers (R1 and R2) were measured and counted; in position A, diameters D1 and D3 were measured; in B (90º of rotation along the longitudinal axis), D2 and D4 were measured. ***(Adapted from Pêgo & Hallauer, 1984)***

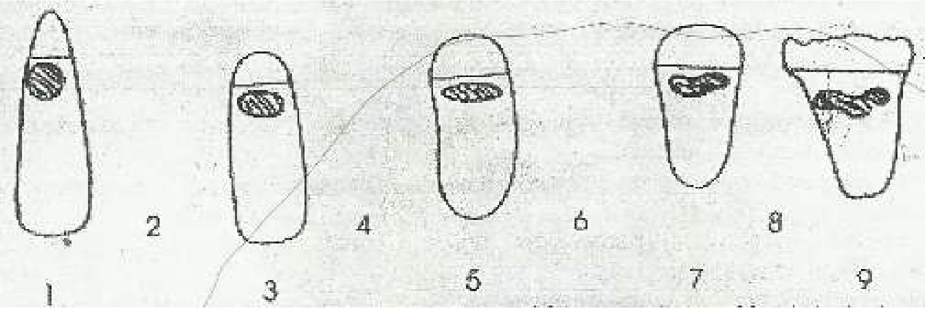


Figure 11- Degree of fasciation (1 - no fasciation and 9 - maximum fasciation), and shape of the ear seen from a cross-section. ***(Adapted from Pêgo & Hallauer, 1984)***

2.7- Statistical methods

To generate descriptive statistics and statistically significant differences, SPSS (Statistical Package for Social) Package 6, version 26 was used. First, morphological parameters and grain yield data were examined using ANOVA (one-way, two-way) to discover statistical differences based on environment, genotype, and trial year. The Tuckey test was then used to distinguish means within each genotype in homogeneous subgroups (SUBSETS). The differences were significant at P<0.05.

To further assess the evolution of yield behavior and identify suitable cultivars for each environment, rank comparisons were performed using a simple rank approach to study overall yield rank behavior in different environments/years.

Furthermore, using the software Past 4.03, Principal Component Analysis (PCA) and biplot analysis were used to classify and identify the genotypes that are more productive in different environments.

Finally, a Pearson correlation was calculated using SPSS (Statistical Package for Social) Package 6, version 26 to assess the statistical relationships between HUNTERS descriptors and ear parameters by grain yield.

2.8- Results and Discussion

2.8.1- Days-to-anthesis (Mi) and days-to-silk (Fi)

The days from sowing to anthesis and silking (Averages) in 50% of the plot individuals of the maize populations (Mi and Fi) are displayed in Figures 12 and 13 respectively.

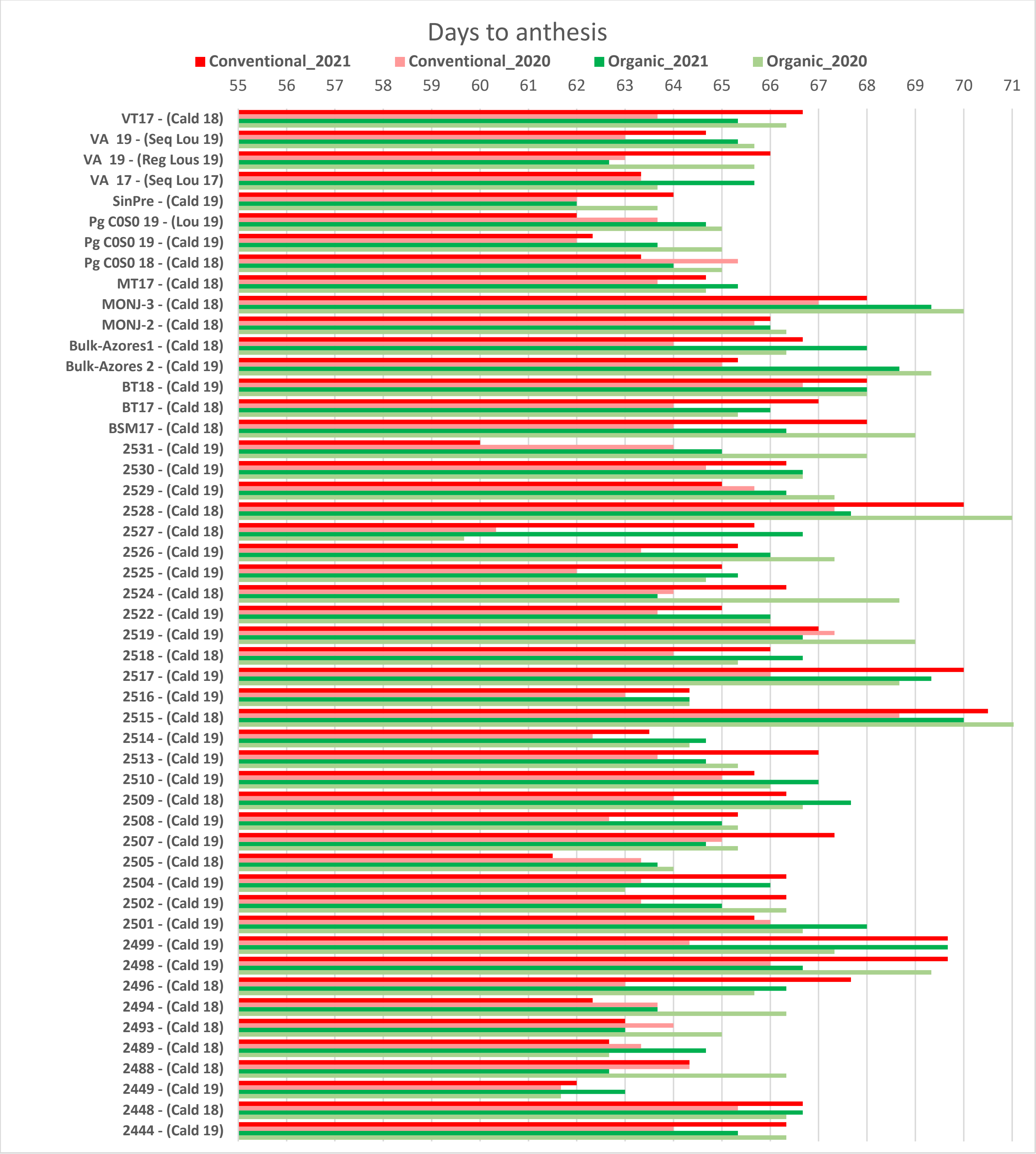


Figure 12- Days to anthesis of the maize populations in the Organic vs Conventional trial from 2020 and 2021

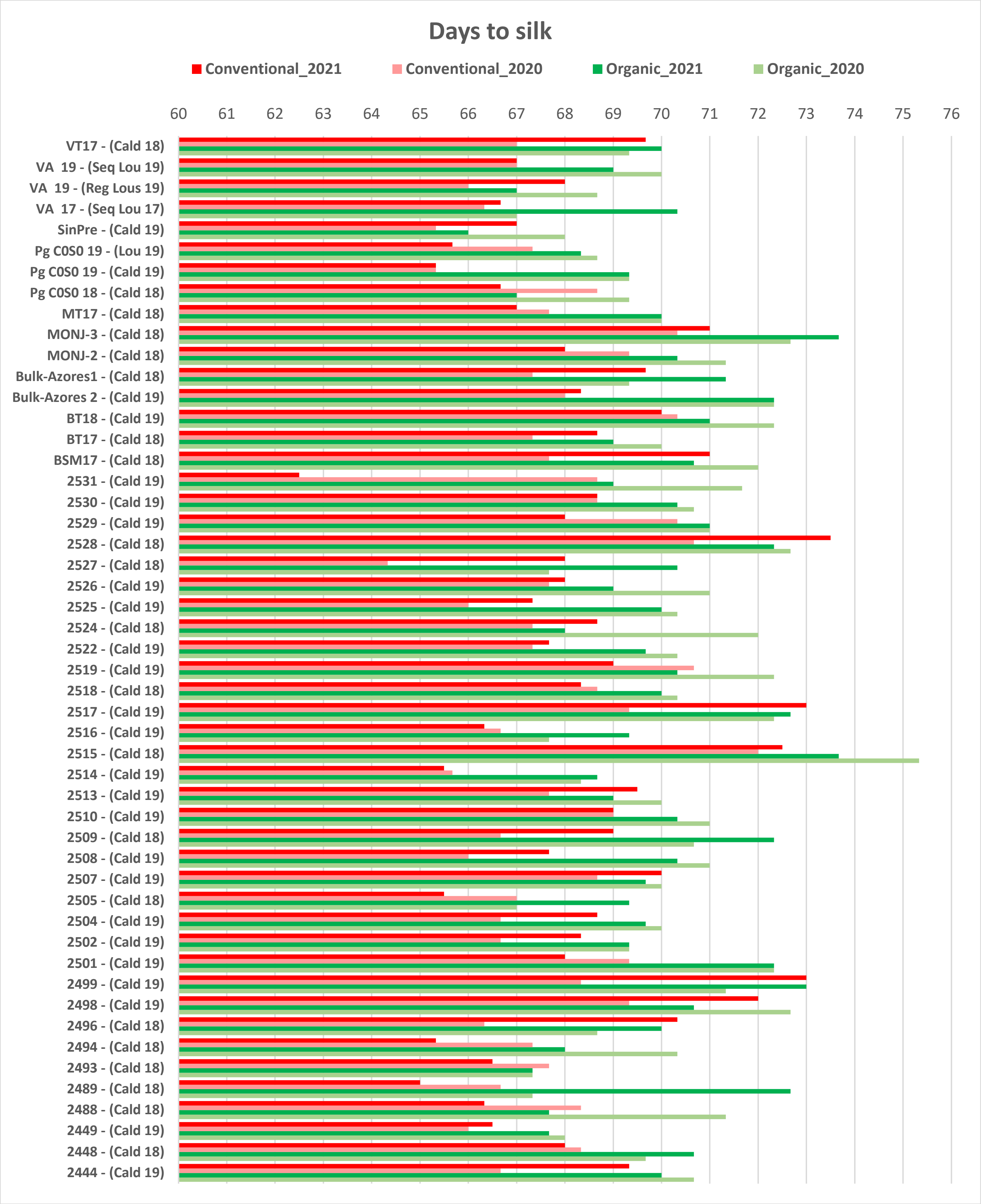


Figure 13- Days to silk of the maize populations in the Organic vs Conventional trial from 2020 and 2021

Afterward, the post hoc test Tuckey identified statistically significant differences within the genotypes and divided were into sub-sets, as shown in Table 9.

Table 9- Averages of Days-to-Anthesis (Mi), Days-to-Silk (Fi), Days-to-Anthesis-end (Mf), Days-to-Silk-end (Fi), and Overlap index (OI) of the maize populations tested (2020-2021). (Letters represent the homogeneous subsets with different significance; Mi: a=0,063, b=0,051, c=0,052, d=0,059, e=0,052, f=0,052, g=0,050, h=0,058; Fi: a=0,088, b=0,103, c=0,056, d=0,056, e=0,056, f=0,056, g=0,096, h=0,066)

Genotype	Mi	Sub-sets Mi	Fi	Sub-sets Fi	Mf	Ff	OI
2444 - (Cald 19)	65,50	a b c d e f g	69,17	a b c d e f g	76,67	84,25	0,74
2448 - (Cald 18)	66,25	a b c d e f g h	69,17	a b c d e f g	77,75	83,58	0,798
2449 - (Cald 19)	62,09	a	67,09	a b	72,82	82,09	0,715
2488 - (Cald 18)	64,42	a b c d e f	68,42	a b c d e f	75,58	82,58	0,788
2489 - (Cald 18)	63,33	a b c	67,92	a b c d e	74,83	83,83	0,723
2493 - (Cald 18)	63,82	a b c d	67,27	a b	75,45	82,82	0,749
2494 - (Cald 18)	64,00	a b c d	67,75	a b c d e	75,33	83,00	0,743
2496 - (Cald 18)	65,67	a b c d e f g	68,83	a b c d e f g	77,08	84,25	0,741
2498 - (Cald 19)	67,92	d e f g h	71,17	d e f g h	79,17	85,92	0,763
2499 - (Cald 19)	67,75	d e f g h	71,42	e f g h	78,50	87,00	0,69
2501 - (Cald 19)	66,58	b c d e f g h	70,50	b c d e f g h	77,75	86,25	0,709
2502 - (Cald 19)	65,25	a b c d e f g	68,42	a b c d e f	76,08	82,75	0,756
2504 - (Cald 19)	64,67	a b c d e f g	68,75	a b c d e f g	75,50	83,33	0,743
2505 - (Cald 18)	63,27	a b c	67,36	a b c	74,27	83,09	0,699
2507 - (Cald 19)	65,58	a b c d e f g	69,58	a b c d e f g	76,50	84,50	0,732
2508 - (Cald 19)	64,58	a b c d e f	68,75	a b c d e f g	75,25	84,58	0,674
2509 - (Cald 18)	66,17	a b c d e f g h	69,67	a b c d e f g	77,50	85,17	0,731
2510 - (Cald 19)	65,92	a b c d e f g	69,83	a b c d e f g h	76,67	84,58	0,729
2513 - (Cald 19)	65,00	a b c d e f g	69,00	a b c d e f g	75,45	83,82	0,706
2514 - (Cald 19)	63,73	a b c d	67,18	a b	74,82	82,55	0,722
2515 - (Cald 18)	70,36	h	73,45	h	81,00	88,55	0,705
2516 - (Cald 19)	64,00	a b c d	67,50	a b c d	75,17	82,08	0,766
2517 - (Cald 19)	68,50	e f g h	71,83	f g h	80,00	87,33	0,742
2518 - (Cald 18)	65,50	a b c d e f g	69,33	a b c d e f g	76,08	84,25	0,709
2519 - (Cald 19)	67,50	c d e f g h	70,58	b c d e f g h	78,33	85,08	0,747
2522 - (Cald 19)	65,17	a b c d e f g	68,75	a b c d e f g	76,17	83,75	0,733
2524 - (Cald 18)	65,67	a b c d e f g	69,00	a b c d e f g	76,92	83,17	0,794
2525 - (Cald 19)	64,25	a b c d e	68,42	a b c d e f	76,17	83,42	0,794
2526 - (Cald 19)	65,50	a b c d e f g	68,92	a b c d e f g	76,75	83,08	0,794
2527 - (Cald 18)	63,08	a b	67,58	a b c d	74,58	82,50	0,771
2528 - (Cald 18)	68,91	g h	72,18	g h	79,73	87,27	0,717
2529 - (Cald 19)	66,08	a b c d e f g	70,08	a b c d e f g h	77,00	84,33	0,766
2530 - (Cald 19)	66,08	a b c d e f g	69,58	a b c d e f g	77,50	85,00	0,741
2531 - (Cald 19)	64,64	a b c d e f g	68,45	a b c d e f	76,00	83,18	0,772
BSM17 - (Cald 18)	66,73	b c d e f g h	70,27	b c d e f g h	77,09	85,64	0,675
BT17 - (Cald 18)	65,58	a b c d e f g	68,75	a b c d e f g	76,50	84,17	0,708
BT18 - (Cald 19)	67,64	d e f g h	71,00	c d e f g h	78,91	86,45	0,729
Bulk-Azores 2 - (Cald 19)	67,08	b c d e f g h	70,25	b c d e f g h	78,42	85,17	0,76
Bulk-Azores1 - (Cald 18)	66,25	a b c d e f g h	69,42	a b c d e f g	77,33	84,58	0,731
MONJ-2 - (Cald 18)	66,00	a b c d e f g	69,75	a b c d e f g	76,92	84,33	0,749
MONJ-3 - (Cald 18)	68,58	f g h	71,92	f g h	79,25	86,67	0,723
MT17 - (Cald 18)	64,58	a b c d e f	68,67	a b c d e f g	76,33	84,75	0,731
Pg 18 - (Cald 18)	64,42	a b c d e f	67,92	a b c d e	76,00	82,67	0,785
Pg 19 - (Cald 19)	63,25	a b c	67,33	a b c	74,92	82,58	0,765
Pg 19 - (Lou 19)	63,83	a b c d	67,50	a b c d	74,67	82,50	0,722
SinPre - (Cald 19)	62,82	a b	66,55	a	73,45	81,82	0,696
VA 17 - (Seq Lou 17)	64,00	a b c d	67,58	a b c d	74,83	82,75	0,714
VA 19 - (Reg Lous 19)	64,33	a b c d e f	67,42	a b c	75,42	82,33	0,743
VA 19 - (Seq Lou 19)	64,67	a b c d e f g	68,25	a b c d e f	75,92	83,25	0,75
VT17 - (Cald 18)	65,50	a b c d e f g	69,00	a b c d e f g	77,33	84,58	0,759

The average values of days to anthesis (Mi) per year of the maize populations ranged from 59.67 (2527 - Cald 18) to 72.33 (2515 - Cald 18) (Figure 12). Maize population 2449-Cald 18 had significantly lower Mi values and maize population 2515- Cald 18 had significantly higher Mi values (Table 9). The mean values of days to silking (Fi) per year of the maize populations ranged from 62.5 (2531- Cald 18) to 75.33 (2515- Cald 18) (Figure 13). The Sinpre-Cald 19 maize population had significantly lower Fi values and the 2515-Cald 18 maize population had significantly higher Fi values (Table 9). Maize population 2515-Cald 18 was tested in 2019 under the same edaphoclimatic conditions (Caldeirão) and showed similar values for Mi and Fi (Duarte Pintado, 2019).

The determination of the overlap index (OI) can predict the relative extent of theoretical allogamy versus autogamy of a maize population, assuming that all pollination occurs under the influence of gravity (Mendes-Moreira et al., 2009). If anthesis and silking overlap in a maize plant, it means that mainly self-pollination occurs. The generally high values (above 0.5) of OI in Table 9 indicate that there is a high risk of inbreeding depression in all maize populations when crossed in a system with free pollination, due to high autogamy (Mendes-Moreira et al., 2009).

2.8.2- HUNTERS descriptor

Average values per entry in terms of plant height (Figure 14) ranged from 181.43 cm (2449- Cald 18) to 300.23 cm (Bulk-Azores 2 - Cald 19), while values in terms of height of first ear insertion (Figure 15) varied from 104.13 cm (VT17- Cald 19) to 194.9 cm (BSM17 - Cald 18). High values for plant height and first ear heigh have been found by other authors (Costa-Rodrigues, 1971; Araújo & Nass, 2002), in the characterization of maize landraces.

Overall, these authors can detect significant differences in plant height and height of the first ear between environments and between genotypes (Tables 10 and 11). As for the different years of the experiment, there were significant differences only in the height of the first ear (Table 12).

*Table 10- One-way ANOVA results on plant height (H) and first ear height (H1E) measurements. The asterisk * indicates statistical differences between the environments (*: P < 0.05; **: P < 0.01; ***: P < 0.001)*

			Sum of Squares	df	Mean Square	F	Sig.
H * Environment	**Between Groups**	**(Combined)**	163431,118	1	163431,118	174,009	,000***
	Within Groups		551316,561	587	939,210		
	Total		714747,679	588			
H1E * Environment	**Between Groups**	**(Combined)**	71647,236	1	71647,236	134,164	,000***
	Within Groups		313474,605	587	534,028		
	Total		385121,841	588			

*Table 11 - One-way ANOVA results on plant height (H) and first ear height (H1E) measurements. The asterisk * indicates statistical differences between the genotypes (*: P < 0.05; **: P < 0.01; ***: P < 0.001)*

			Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
H * Genotype	**Between Groups**	**(Combined)**	89050,282	49	1817,353	1,566	,010*
	Within Groups		625697,397	539	1160,849		
	Total		714747,679	588			
H1E * Genotype	**Between Groups**	**(Combined)**	85072,439	49	1736,172	3,119	,000***
	Within Groups		300049,402	539	556,678		
	Total		385121,841	588			

*Table 12- - One-way ANOVA results on plant height (H) and first ear height (H1E) measurements. The asterisk * indicates statistical differences between the years of the trial (*: P < 0.05; **: P < 0.01; ***: P < 0.001)*

			Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
H * Year	**Between Groups**	**(Combined)**	,486	1	,486	,000	,984
	Within Groups		714747,193	587	1217,627		
	Total		714747,679	588			
H1E * Year	**Between Groups**	**(Combined)**	6855,203	1	6855,203	10,638	,001**
	Within Groups		378266,638	587	644,407		
	Total		385121,841	588			

Following that, the Tuckey post hoc test revealed statistically significant differences between genotypes and separated them into subgroups (SUBSET), as displayed next to the genotype name in Figures 14 and 15. The Azorean populations VT17- (Cald 18) and 2449- (Cald 18) showed statistically significant smaller plants and the Azorean population BSM17 - (Cald 18) showed significantly taller plants, displaying the great diversity in height present in the Azorean germplasm. Plant height (H) indicates significantly taller maize plants in the conventional environment. Two maize populations (2501 - Cald 18 and 2493 - Cald 18) had average taller plants in the organic environment than in the conventional environment in 2021 (Figure 14), showing an interesting level of adaptation in terms of biomass production (silage suitability) in the low input organic environment.

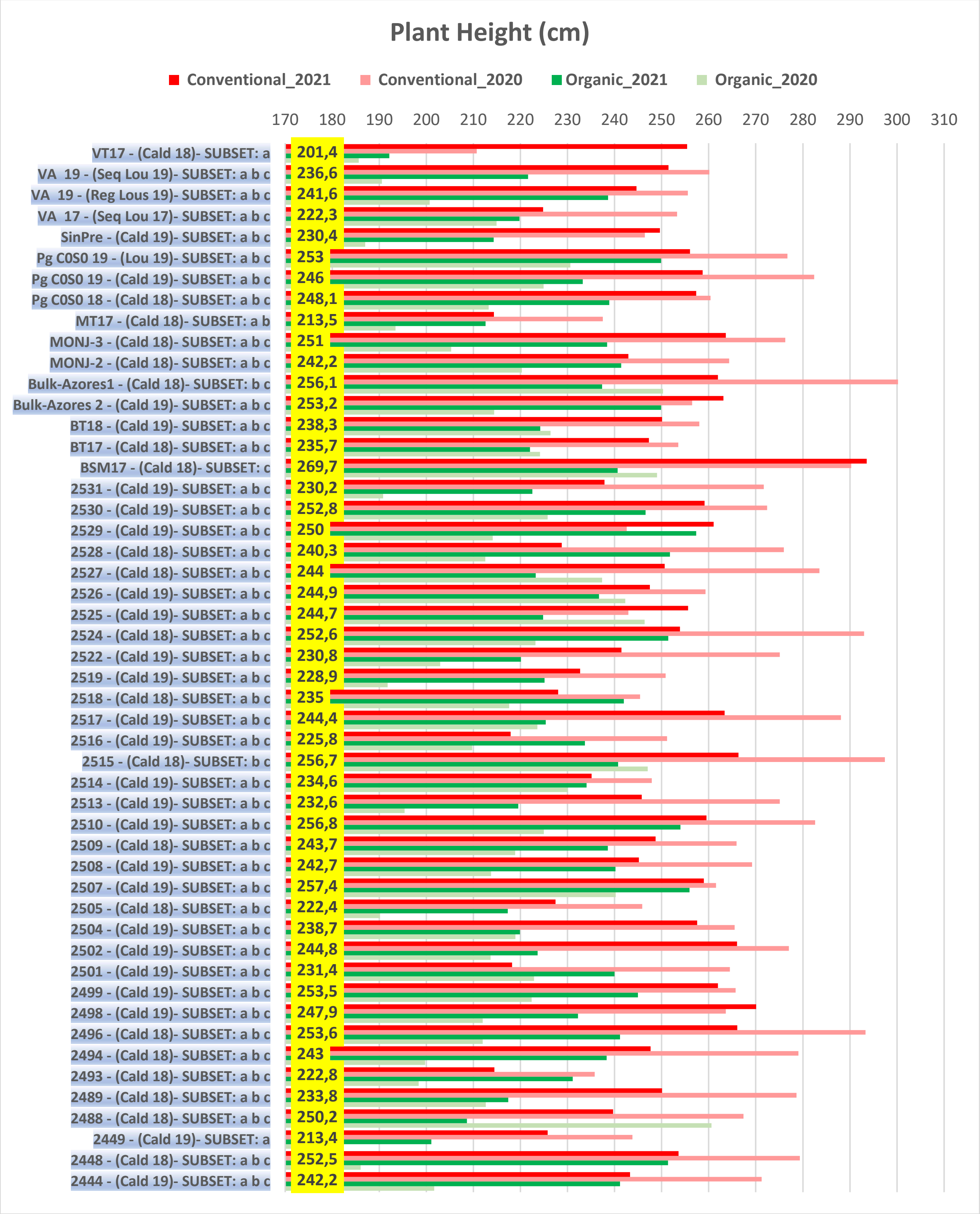


Figure 14- Plant height (H) average values of the genotypes by environment and year (bars) and Plant height (H) overall average values of the trial (values). SUBSET- represent the homogeneous subsets (Tukey's multiple comparisons test) with different significance; a=0,080, b=0,090, c=0,129

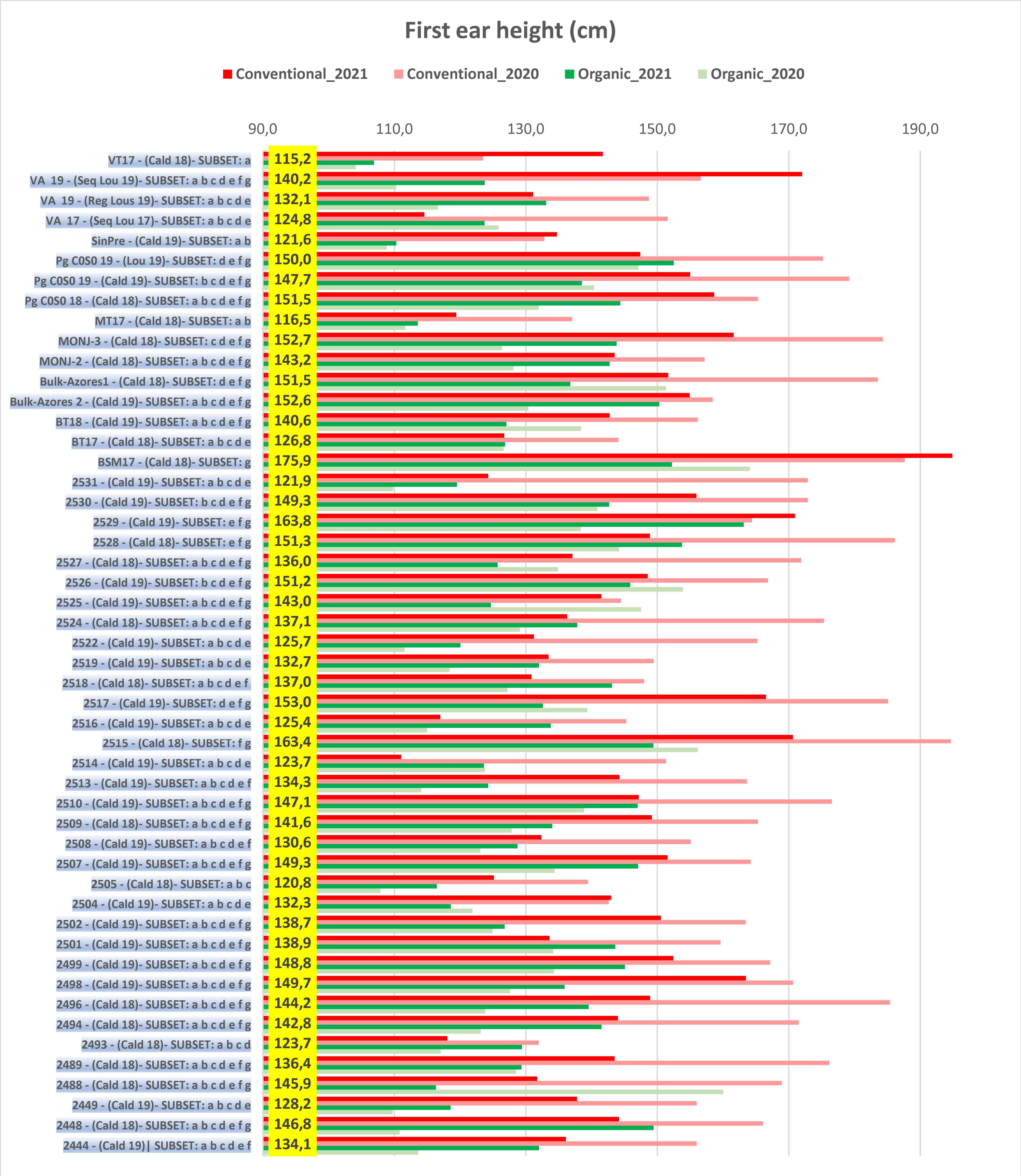


Figure 15- First ear height (H1e) average values of the genotypes by environment and year (bars) and first ear height (H1e) overall average values of the trial (values). SUBSET- represent the homogeneous subsets (Tukey's multiple comparisons test) with different significance; a=0,066, b=0,059, c=0,051, d=0,132, e=0,071, f=0,074, g=0,066).

The average genotype values related to the uniformity parameter (U) (Table 15) show that most maize populations reached values between 3 and 3.99 (76%), 10 maize populations had values between 2 and 2.99 (20%), and only 2529 - (Cald 19) and BSM17 - (Cald 18) had uniformity values above 4. These uniformity values indicate a high genetic diversity within populations, which is beneficial for plant adaptation and breeding .(Costa-Rodrigues, 1971; Vaz Patto et al., 2008). In addition, significant differences were found between environments. Organic Caldeirão (2.38) achieved significantly lower average U values than Conventional Vagem (3.16) (Table 13). The Organic environment generates greater variability in the way plants respond to abiotic stress, highlighting the need to test and improve cultivars in organic environments.

*Table 13- One-way ANOVA results on Uniformity values. The asterisk * indicates statistical differences between the environments (Organic vs Conventional) (*: P < 0.05; **: P < 0.01; ***: P < 0.001)*

			Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
U * Environment	**Between Groups**	**(Combined)**	89,303	1	89,303	168,900	,000***
	Within Groups		309,307	585	,529		
	Total		398,610	586			

According to the collected data on leaf angle (N) (Table 15), the average values of the genotypes ranged from 5 (2499 - Cald 19) to 7 (2505 - Cald 19). The higher N values indicate that the tested maize populations were grown in polyculture systems with low seeding densities (Mendes-Moreira et al., 2017; Moreira, 2006). The leaf angle values not only showed adaptation to this type of system used in Portuguese family farming, but also represent a tool to control the problem of high exposure/shade in relation to the associated crop in the polyculture system (Mendes-Moreira et al., 2017; Pêgo, 1982). Significant differences were also found between environments. Organic Caldeirão (6.30) reached significantly higher average N values than Conventional Vagem (5.46) (Table 14). This can be explained by the lower biomass produced in the organic environment, resulting in more space for maize plants to develop this trait.

*Table 14- One-way ANOVA results about Leaf angle values (N). The asterisk * indicates statistical differences between the environments (Organic vs Conventional) (*: P < 0.05; **: P < 0.01; ***: P < 0.001)*

			Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
N * Environment	**Between Groups**	**(Combined)**	105,200	1	105,200	121,002	,000***
	Within Groups		508,602	585	,869		
	Total		613,802	586			

The evaluation of the maize populations tassel (T) (Table 15) shows that overall they are large and very branched, only 10% of the maize populations have values below 6. Recent literature (Silveira et al., 2021), indicates that large tassels with a higher number of branches prevent the passage of solar radiation into the upper plant canopy and serve as an outflow for photoassimilates. However, maize de-tasseling was an agronomic practice commonly performed by Portuguese farmers, and the biomass produced was usually used as feed for livestock.

A closer look at the values for first ear insertion (E) (Table 15) shows that only one maize population (2493 - Cald 18) scored below 5, indicating that most of the maize populations tested had ears set above midplant. For maize populations with E values above 6 (8%), manual harvest can be challenging.

Combining all the phenotypic data from the field trials helped to show the tremendous variability within the maize populations tested. Similar phenological values were found in the 2019 field trials with 20 Azorean landraces included in this trial (Duarte Pintado, 2019). It also displays information that can help farmers formulate their product philosophy and field management. The unique variety presented can be grown in sustainable and organic environments and meets many needs (animal feed and human food) due to its strong gastronomic potential already reported (Duarte Pintado, 2019; Vaz Patto et al., 2008).

Table 15-Averages and Standard deviations referred to the HUNTERS parameters (Uniformity, Leaf Angle, Tassel and Ear placement), used to characterize the maize genotypes of the Organic Vs Conventional trial

Genotype	U	N	T	E
2444 - (Cald 19)	3,67 ± 0,58	5,33 ± 0,577	6,67 ± 0,58	5 ± 0
2448 - (Cald 18)	2,67 ± 0,58	6,67 ± 0,577	5,33 ± 0,58	5,33 ± 0,58
2449 - (Cald 19)	3 ± 0	5,5 ± 0,707	7 ± 0,71	5,5 ± 0,71
2488 - (Cald 18)	2,67 ± 0,58	6,33 ± 0,577	6 ± 0,58	5,33 ± 0,58
2489 - (Cald 18)	2,33 ± 0,58	6 ± 2,646	6,33 ± 2,65	5,33 ± 0,58
2493 - (Cald 18)	3,5 ± 0,71	5,5 ± 0,707	7 ± 0,71	4,5 ± 0,71
2494 - (Cald 18)	3 ± 0	6 ± 1	6,67 ± 1	5,33 ± 0,58
2496 - (Cald 18)	3,33 ± 1,53	6 ± 1	7 ± 1	5,67 ± 0,58
2498 - (Cald 19)	3,33 ± 1,16	5 ± 1	7,33 ± 1	5,67 ± 0,58
2499 - (Cald 19)	3 ± 1,41	5 ± 1,414	6,5 ± 1,41	6 ± 0
2501 - (Cald 19)	3,33 ± 0,58	5,33 ± 0,577	6 ± 0,58	5,33 ± 0,58
2502 - (Cald 19)	3,67 ± 0,58	5,33 ± 1,528	6 ± 1,53	5,67 ± 0,58
2504 - (Cald 19)	3,67 ± 0,58	6 ± 1	6 ± 1	5,33 ± 0,58
2505 - (Cald 18)	3,5 ± 0,71	7 ± 0	7 ± 0	5 ± 0
2507 - (Cald 19)	3,67 ± 0,58	6,33 ± 1,155	6,33 ± 1,16	5 ± 0
2508 - (Cald 19)	2,67 ± 0,58	6,33 ± 1,528	6,33 ± 1,53	5,33 ± 0,58
2509 - (Cald 18)	3 ± 1	6 ± 1	7 ± 1	5,67 ± 0,58
2510 - (Cald 19)	3,33 ± 0,58	5,67 ± 0,577	6,67 ± 0,58	5,67 ± 0,58
2513 - (Cald 19)	3,5 ± 0,71	5 ± 0	6,5 ± 0	5 ± 0
2514 - (Cald 19)	3 ± 0	6,5 ± 0,707	4,5 ± 0,71	5 ± 0
2515 - (Cald 18)	3 ± 1,41	5 ± 1,414	6,5 ± 1,41	5,5 ± 0,71
2516 - (Cald 19)	3 ± 0	6,33 ± 0,577	5,67 ± 0,58	5,33 ± 0,58
2517 - (Cald 19)	3,33 ± 1,16	5 ± 1	6 ± 1	5,33 ± 0,58
2518 - (Cald 18)	3,33 ± 0,58	6,33 ± 1,155	6 ± 1,16	5 ± 0
2519 - (Cald 19)	3,33 ± 1,16	5,33 ± 1,528	7 ± 1,53	5,33 ± 0,58
2522 - (Cald 19)	3 ± 1	5,67 ± 2,082	6,67 ± 2,08	5,33 ± 0,58
2524 - (Cald 18)	2,67 ± 0,58	6,33 ± 0,577	5,67 ± 0,58	5,33 ± 0,58
2525 - (Cald 19)	2,67 ± 0,58	6,33 ± 0,577	6,33 ± 0,58	5,67 ± 0,58
2526 - (Cald 19)	3 ± 1	5,67 ± 0,577	6,33 ± 0,58	5,33 ± 0,58
2527 - (Cald 18)	2,67 ± 1,16	6 ± 1,732	6 ± 1,73	5,67 ± 0,58
2528 - (Cald 18)	2,5 ± 0,71	4,5 ± 0,707	5 ± 0,71	5,5 ± 0,71
2529 - (Cald 19)	4 ± 0	5,33 ± 0,577	6,67 ± 0,58	5,33 ± 0,58
2530 - (Cald 19)	3,67 ± 0,58	6 ± 1	6,67 ± 1	5,33 ± 0,58
2531 - (Cald 19)	3,5 ± 0,71	6 ± 0	6 ± 0	5,5 ± 0,71
BSM17 - (Cald 18)	4 ± 0	7 ± 0	7 ± 0	6 ± 0
BT17 - (Cald 18)	2,67 ± 0,58	6,33 ± 1,528	7 ± 1,53	5,67 ± 0,58
BT18 - (Cald 19)	3 ± 1,41	5,5 ± 0,707	5,5 ± 0,71	5 ± 0
Bulk-Azores 2 - (Cald 19)	3,67 ± 0,58	5,67 ± 1,528	6 ± 1,53	5,33 ± 0,58
Bulk-Azores1 - (Cald 18)	2,67 ± 0,58	6,33 ± 0,577	6 ± 0,58	5,67 ± 0,58
MONJ-2 - (Cald 18)	3,67 ± 0,58	6 ± 1	5,33 ± 1	6 ± 0
MONJ-3 - (Cald 18)	3,5 ± 0,71	6 ± 1,414	6 ± 1,41	6,5 ± 0,71
MT17 - (Cald 18)	3 ± 0	6 ± 1	6 ± 1	5,33 ± 0,58
Pg 18 - (Cald 18)	3,67 ± 0,58	5,67 ± 1,528	6,67 ± 1,53	5,33 ± 0,58
Pg 19 - (Cald 19)	3 ± 1	5,33 ± 0,577	6,67 ± 0,58	5,67 ± 1,16
Pg 19 - (Lou 19)	2,67 ± 0,58	6 ± 1	6,67 ± 1	5,67 ± 0,58
SinPre - (Cald 19)	3,5 ± 0,71	6 ± 0	6,5 ± 0	5,5 ± 0,71
VA 17 - (Seq Lou 17)	3,67 ± 0,58	4,67 ± 0,577	5 ± 0,58	5 ± 0
VA 19 - (Reg Lous 19)	2,67 ± 0,58	5,33 ± 1,528	6,33 ± 1,53	5,33 ± 0,58
VA 19 - (Seq Lou 19)	2,67 ± 0,58	5,67 ± 0,577	6 ± 0,58	4,67 ± 0,58
VT17 - (Cald 18)	2,67 ± 0,58	5,67 ± 0,577	6,33 ± 0,58	5,67 ± 0,58

Due to a storm in September 2021, the values of Root lodging (R) and Stalk lodging (S) were compromised. The plants were lodged due to the strong wind and rain. Nevertheless, the 2020 data showed significant differences in Root lodging between genotypes and environment (Table 16 and 17). Stalk lodging averages reveal significant differences in environment (Table 17).

The Conventional Vagem due to higher fertilization and consequent higher biomass production, reveal significantly higher Stalk lodging values (Table 16). Some authors argue that the weight of the ear can increase the risk of lodging when mixed with a higher ear position (Xue et al., 2020).

The Organic Caldeirão showed significantly higher average values of Root lodging mainly due to its proximty with the Mondego River and its lower position about the surrounding land, which poses a risk of waterlogging in the final month of the maize cycle, providing conditions to the development of root diseases.

*Table 16- One-way ANOVA results about Root lodging (R) and Stalk lodging (S) values. The asterisk * indicates statistical differences between the genotypes (*: P < 0.05; **: P < 0.01; ***: P < 0.001)*

			Sum of Squares	df	Mean Square	F	Sig.
%R * Genotype	**Between Groups**	**(Combined)**	24562,505	49	501,276	1,537	,019*
	Within Groups		81514,193	250	326,057		
	Total		106076,699	299			
%S * Genotype	**Between Groups**	**(Combined)**	4995,426	49	101,947	,859	,734
	Within Groups		29666,912	250	118,668		
	Total		34662,338	299			

*Table 17- One-way ANOVA results about Root lodging (R) and Stalk lodging (S) values. The asterisk * indicates statistical differences between the environments (Organic vs Conventional) (*: P < 0.05; **: P < 0.01; ***: P < 0.001)*

			Sum of Squares	df	Mean Square	F	Sig.
%R * Environment	**Between Groups**	**(Combined)**	4313,774	1	4313,774	12,632	,000***
	Within Groups		101762,925	298	341,486		
	Total		106076,699	299			
%S * Environment	**Between Groups**	**(Combined)**	1931,049	1	1931,049	17,581	,000***
	Within Groups		32731,289	298	109,837		
	Total		34662,338	299			

Afterward, the post hoc test Tuckey identified statistically significant differences within the genotypes that only occur in Root lodging (R) (Table 16), and divided were into subgroups (SUBSET-displayed next to the genotype name) as shown in the graph representation in Figure 16.

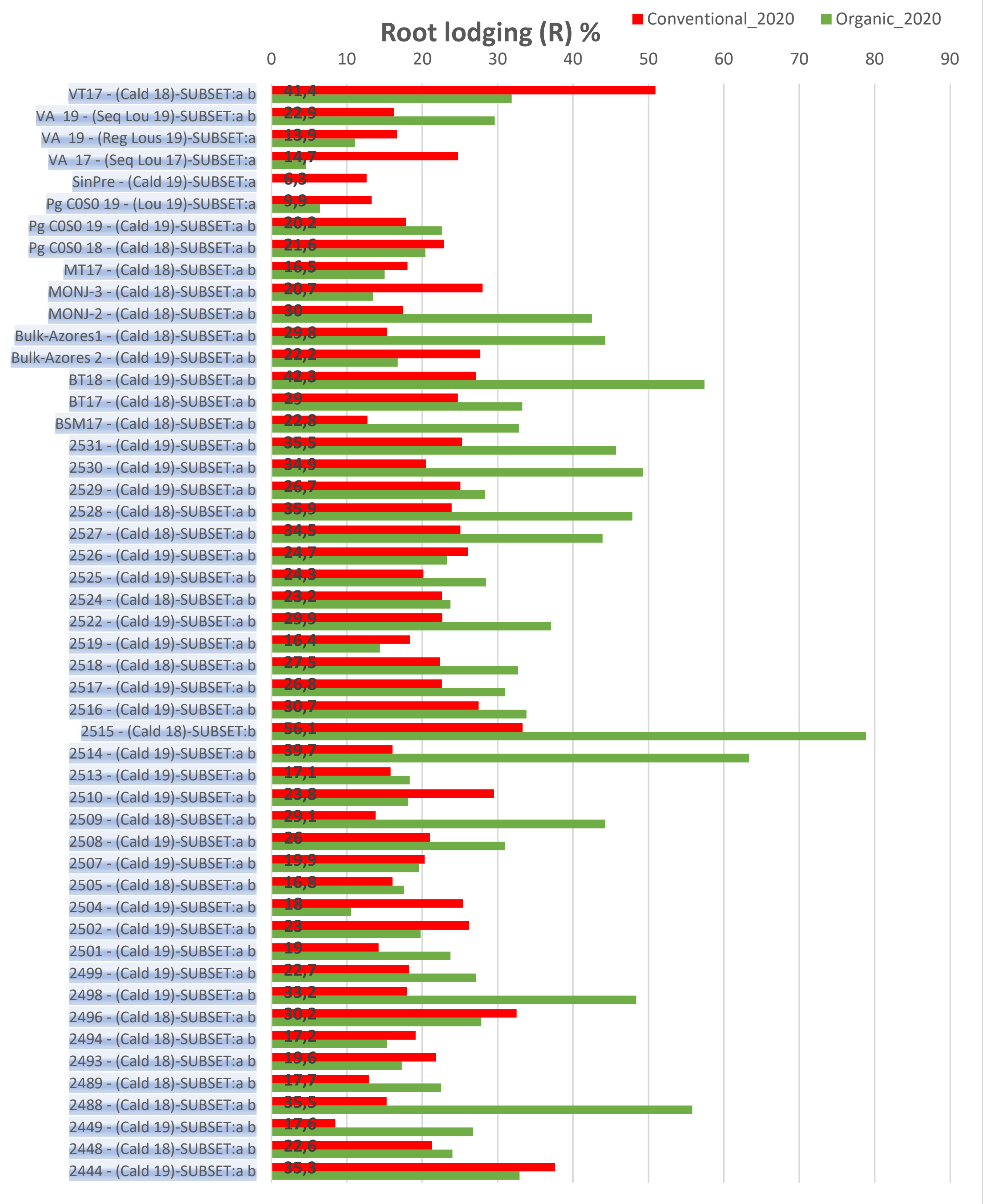


Figure 16- Root lodging (R) average values of the genotypes by environment and year (bars) and Root lodging (R) overall average values of 2020 (values). SUBSET- represent the homogeneous subsets (Tukey's multiple comparisons test) with different significance; a=0,080, b=0,090.

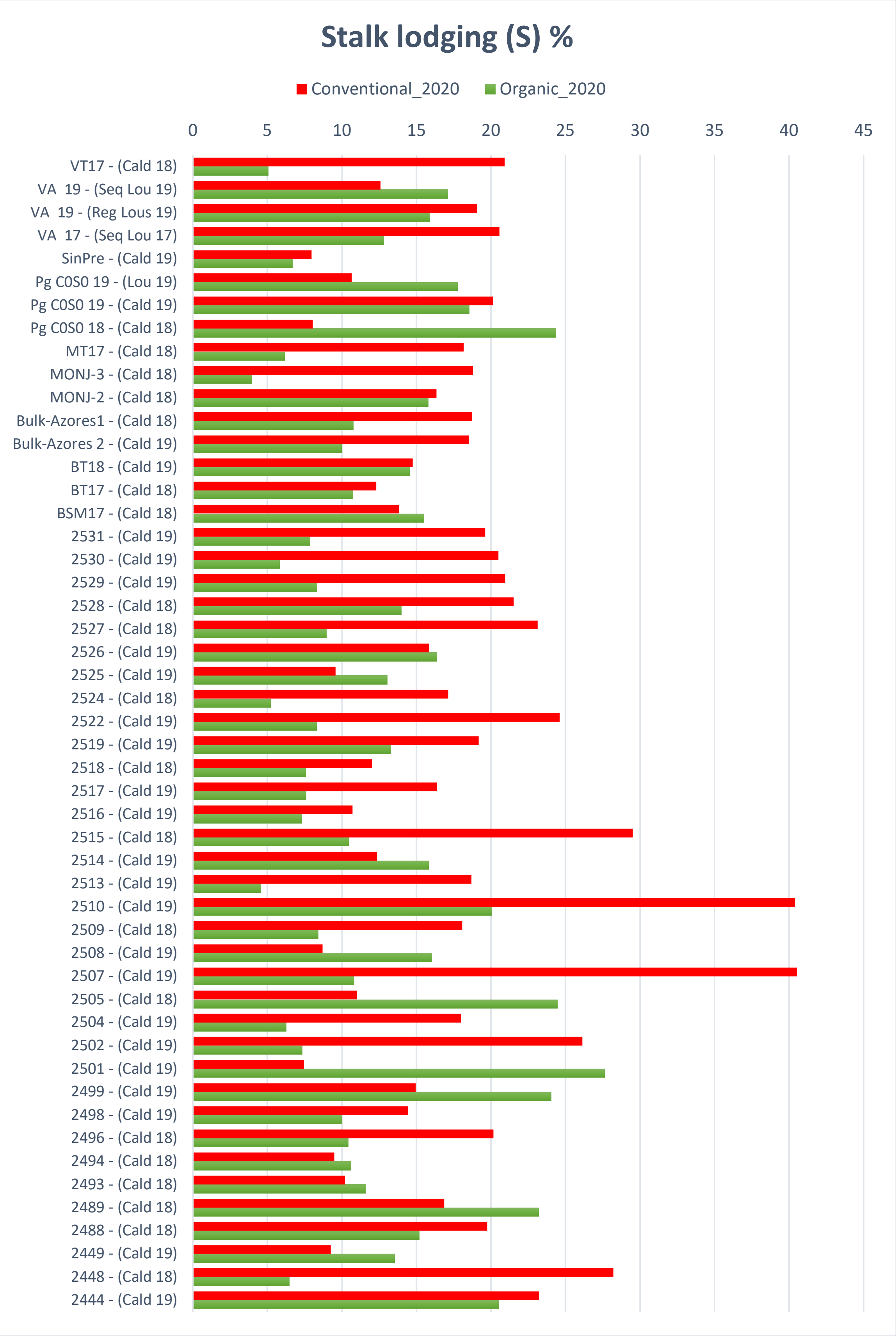


Figure 17- Average genotype values of Stalk lodging (S) of the Organic Vs Conventional trial in 2020.

We can highlight three populations whose Root Lodging (R) values were significantly lower: VA 17- (Seq Lou 17), Pg 19- (Lou 19), and Sinpre- (Cald 19). These three populations originated from a breeding programme (VASO), which explains their significantly lower values in this parameter compared to the Azorean populations. The maize population Sinpre- (Cald 19) showed the best behaviour in the Organic environment, with no plants leaning more than 30º from the vertical in all three repetitions (plots).

2.8.3- Grain Yield evaluations

Mean grain yields ranged from 1 116.4 kg/ha to 9 223.3 kg/ha, with statistically significantly higher values in Conventional Vagem 2020.

ANOVA analysis revealed significant differences between average grain yield values among genotype, environment, year, and environment by year (Table 18). Next, the post hoc Tuckey test identified statistically significant differences within genotypes and divided them into subgroups (SUBSET-displayed next to the genotype name) as shown in Figure 18.

*Table 18- Two-way ANOVA results about the statistical interaction between; Genotype, environment, and year by grain yield averages. The asterisk * indicates statistical interaction (*: P<0.05; **: P<0.01; ***: P<0.001)*

Dependent Variable: Grain Yield					
Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Corrected Model	1930866947,205^a	199	9702848,981	4,694	,000
Intercepto	8686031360,665	1	8686031360,665	4202,003	,000
Environment	1026151986,271	1	1026151986,271	496,417	,000***
Genotype	175498827,618	49	3581608,727	1,733	,003***
Year	335049058,082	1	335049058,082	162,085	,000***
Environment * Genotype	120674814,254	49	2462751,311	1,191	,187
Environment * Year	33578939,883	1	33578939,883	16,244	,000***
Genotype * Year	124085582,303	49	2532358,823	1,225	,153
Environment * Genotype * Year	82547439,697	49	1684641,626	,815	,808
Error	804108468,148	389	2067116,885		
Total	11451103387,101	589			
Corrected Total	2734975415,353	588			
a. R Squared = ,706 (Adjusted R Squared = ,556)					

When looking at the overall grain yield values, two populations stand out with significantly higher values: Pg 19- (Lou 19) and 2496- (Cald 18) (Figure 18).

Regarding only the organic environment, there were no significant differences among genotypes, but there were significantly lower average values in 2021 (Table 19), mainly due to the previously reported storm.

*Table 19- One-way ANOVA results about the interaction between Grain Yield (Organic Caldeirão) and the year of the trial. The asterisk * indicates statistical interaction (*: P<0.05; **: P<0.01; ***: P<0.001)*

			Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Grain yield (organic) * Year	**Between Groups**	**(Combined)**	80396933,015	1	80396933,015	76,958	,000***
	Within Groups		311314722,298	298	1044680,276		
	Total		391711655,313	299			

Grain yield values were subjected to a simple ranking (Table 20) to explain the global production behaviour during the two experimental years in each environment. A detailed look at Table 20 reveals the best grain yield values (top 3) in the two different environments (Organic Caldeirão and Conventional Vagem) and we can identify genotypes 2516 - (Cald 19), 2501 - (Cald 19) and 2507 - (Cald 19) with the best organic behaviour. However, in the conventional environment, genotypes 2496 - (Cald 18), 2448 - (Cald 18) and 2499 - (Cald 19) showed the best productive behaviour as the top 3.

In the first year of the trial, the 3 most productive maize populations in Organic Caldeirão were Bulk-Azores 2 - (Cald 19), VA 19 - (Reg Lou 19) and 2501- (Cald 19). The waterlogging combined with strong winds in the second year of the experiment (2021) in the Organic environment revealed the resistant behavior of the Azorean population 2516 - (Cald 19) and we can identify it as the most suitable for marginal low input organic environments.

Highlights to the good behavior of the Azorean maize populations that, not being targeted by participatory breeding before, achieved similar values in the organic environment compared to maize populations originated by PPB VASO. The full potential of these maize populations can be explored and represents proof that there is viability in the adaptation of genetic resources to organic agriculture.

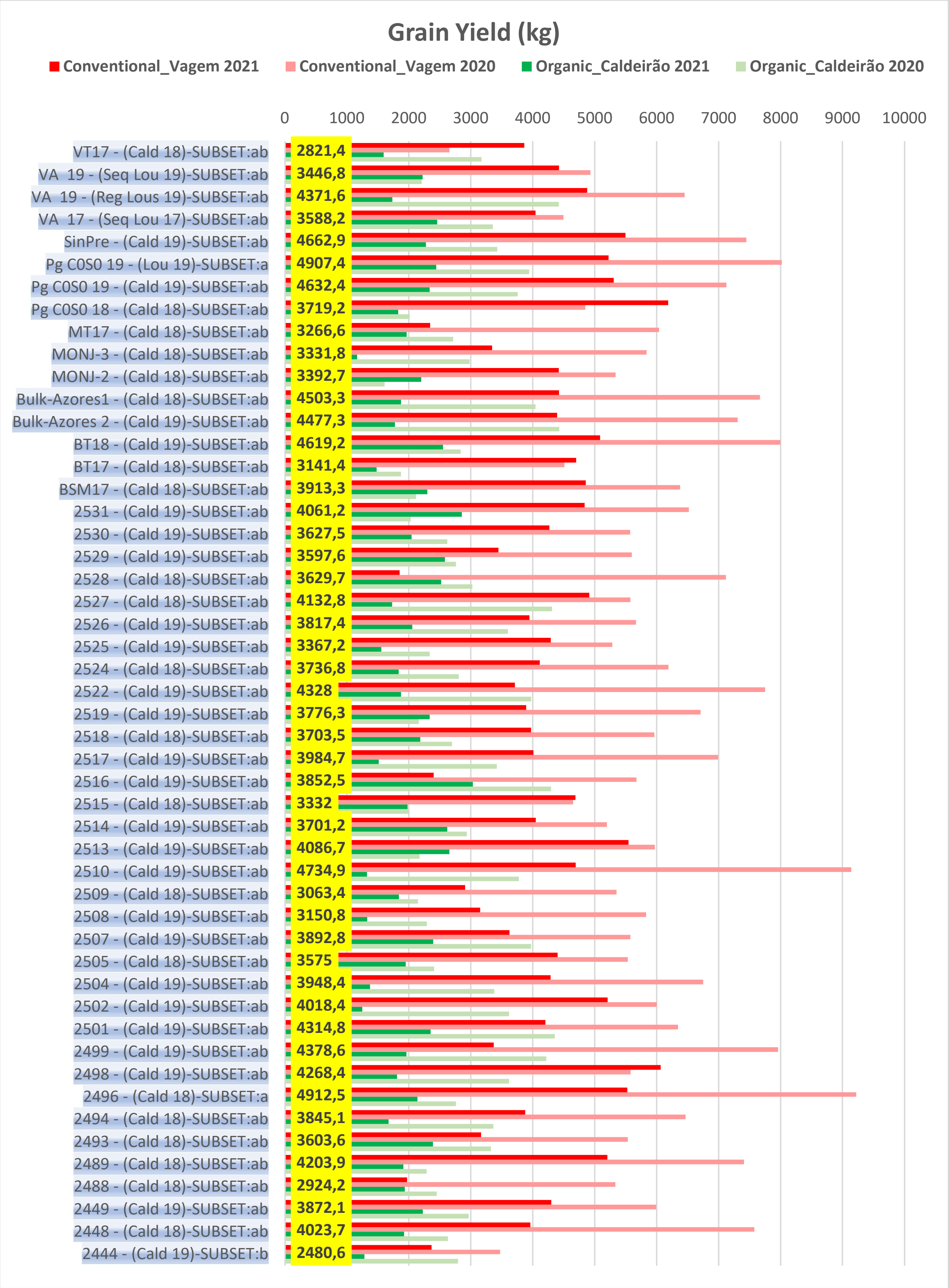


Figure 18- Grain yield average values of the genotypes by environment and year (bars) and Grain yield overall average values of the trial (values). SUBSET- represent the homogeneous subsets (Tukey's multiple comparisons test) with different significance; a=0,05, b=0,090

*Table 20- The overall Organic Grain Yield ranking of the Organic vs Conventional trial **(OC20- Organic Caldeirão 2020; OC21- Organic Caldeirão 2021; CV20- Conventional Vagem 2020; CV21- Conventional Vagem 2021)***

Genotype	Rank OC 20	Rank OC21	∑ OC	Rank OC	Rank CV20	Rank CV21	∑ CV	Rank CV	∑ Total	Rank Total
2516 - (Cald 19)	5	1	6	1	30	22	52	30	58	3
2501 - (Cald 19)	3	12	15	2	21	11	32	9	47	1
2507 - (Cald 19)	8	10	18	3	34	15	49	23	67	5
Pg 19 - (Lousada)	10	9	19	4	3	45	48	22	67	6
Pg 19 - (Cald 19)	12	14	26	5	12	44	56	33	82	14
VA 17 - (Seq Lou 2017)	20	8	28	6	48	47	95	49	123	38
2528 - (Cald 18)	23	7	30	7	13	31	44	16	74	10
2514 - (Cald 19)	26	4	30	8	43	20	63	36	93	23
2499 - (Cald 19)	6	26	32	9	5	10	15	3	47	2
2493 - (Cald 18)	21	11	32	10	38	6	44	17	76	12
SinPre - (Cald 19)	16	16	32	11	9	46	55	31	87	19
BT18 - (Cald 19)	27	6	33	12	4	37	41	13	74	11
2529 - (Cald 19)	31	5	36	13	32	32	64	37	100	29
2526 - (Cald 19)	15	22	37	14	31	29	60	35	97	24
Bulk-Azores 2 - (Cald 19)	1	37	38	15	11	38	49	24	87	18
Bulk-Azores1 - (Cald 18)	7	32	39	16	7	39	46	21	85	17
2522 - (Cald 19)	9	31	40	17	6	26	32	10	72	8
VA 19 - (Reg Lou 19)	2	38	40	18	19	48	67	40	107	31
2449 - (Cald 19)	25	17	42	19	25	3	28	7	70	7
2527 - (Cald 18)	4	39	43	20	35	30	65	38	108	32
2513 - (Cald 19)	42	3	45	21	26	19	45	18	90	20
2531 - (Cald 19)	46	2	48	22	17	34	51	27	99	28
2498 - (Cald 19)	13	36	49	23	33	9	42	15	91	21
2496 - (Cald 18)	30	21	51	24	1	8	9	1	60	4
2518 - (Cald 18)	33	20	53	25	27	24	51	28	104	30
2519 - (Cald 19)	43	13	56	26	16	25	41	14	97	26
MT17 - (Cald 18)	32	25	57	27	23	42	65	39	122	37
2510 - (Cald 19)	11	47	58	28	2	18	20	5	78	13
2530 - (Cald 19)	35	23	58	29	36	33	69	42	127	39
2494 - (Cald 18)	19	40	59	30	18	7	25	6	84	15
VA 19 - (Seq Lou 19)	41	18	59	31	44	49	93	48	152	47
2517 - (Cald 19)	17	43	60	32	14	23	37	12	97	25
BSM17 - (Cald 18)	45	15	60	33	20	35	55	32	115	35
2524 - (Cald 18)	28	34	62	34	22	27	49	25	111	34
2448 - (Cald 18)	34	29	63	35	8	2	10	2	73	9
2504 - (14-2019 Cald 19)	18	45	63	36	15	13	28	8	91	22
2502 - (Cald 19)	14	49	63	37	24	12	36	11	99	27
VT17 - (Cald 18)	22	41	63	38	50	50	100	50	163	48
2488 - (Cald 18)	36	28	64	39	41	4	45	19	109	33
2505 - (Cald 18)	37	27	64	40	37	14	51	29	115	36
MONJ-2 - (Cald 18)	50	19	69	41	40	40	80	45	149	45
2489 - (Cald 18)	40	30	70	42	10	5	15	4	85	16
2515 - (Cald 18)	48	24	72	43	46	21	67	41	139	43
MONJ-3 - (Cald 18)	24	50	74	44	28	41	69	43	143	44
2444 - (Cald 19)	29	48	77	45	49	1	50	26	127	40
2509 - (Cald 18)	44	33	77	46	39	17	56	34	133	42
2525 - (Cald 19)	38	42	80	47	42	28	70	44	150	46
Pg 18 - (Cald 18)	47	35	82	48	45	43	88	47	170	49
2508 - (Cald 19)	39	46	85	49	29	16	45	20	130	41
BT17 - (Cald 18)	49	44	93	50	47	36	83	46	176	50

Figures 19 and 20 represent a PCA analysis and biplot analysis of grain yield values from both years of the trial.

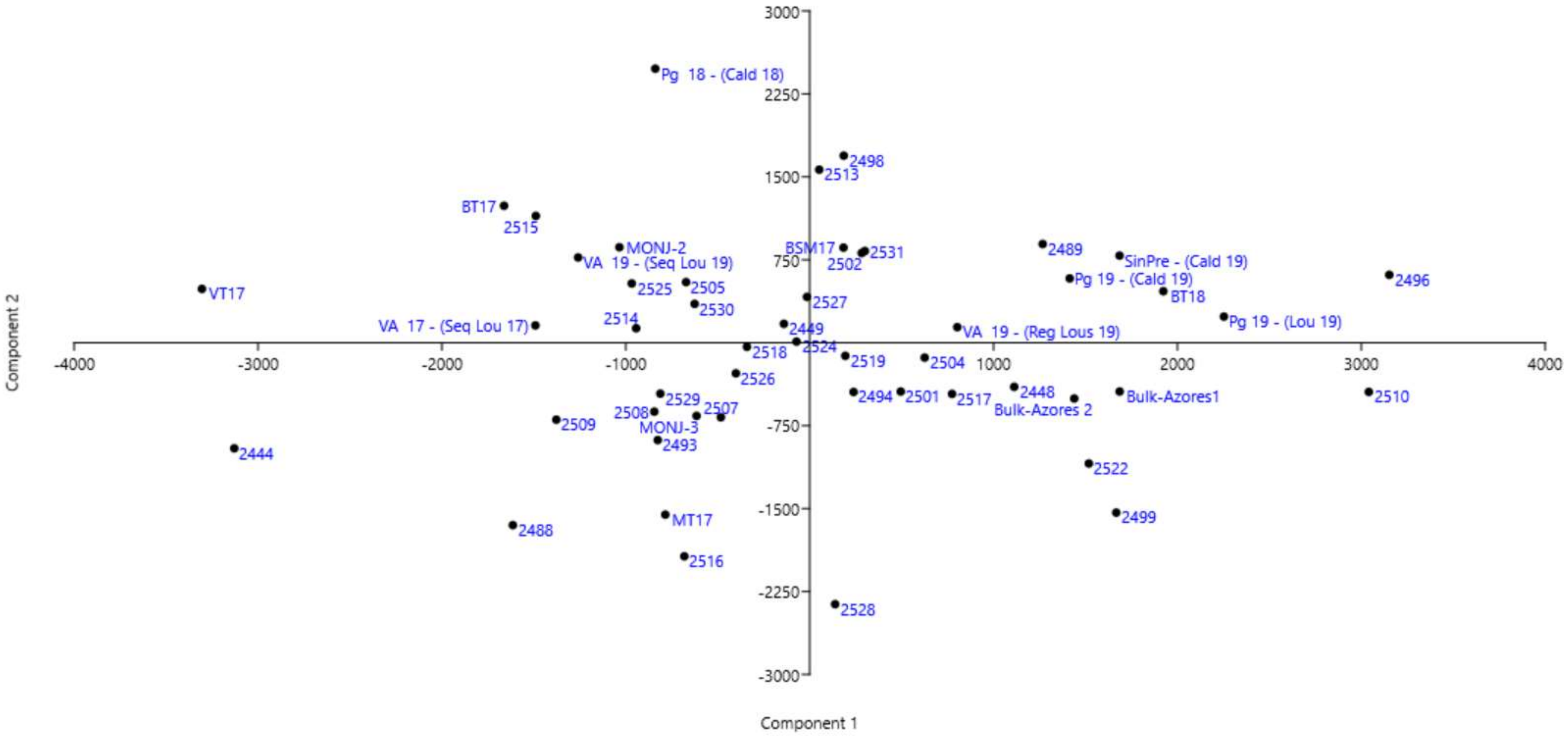


Figure 19- The results of a PCA analysis of the genotypes' average grain yield values in the Organic vs. Conventional trial in 2020 and 2021. (Components 1 and 2 explain 53,7 % and 26,46 %. of the variance detected, respectably)

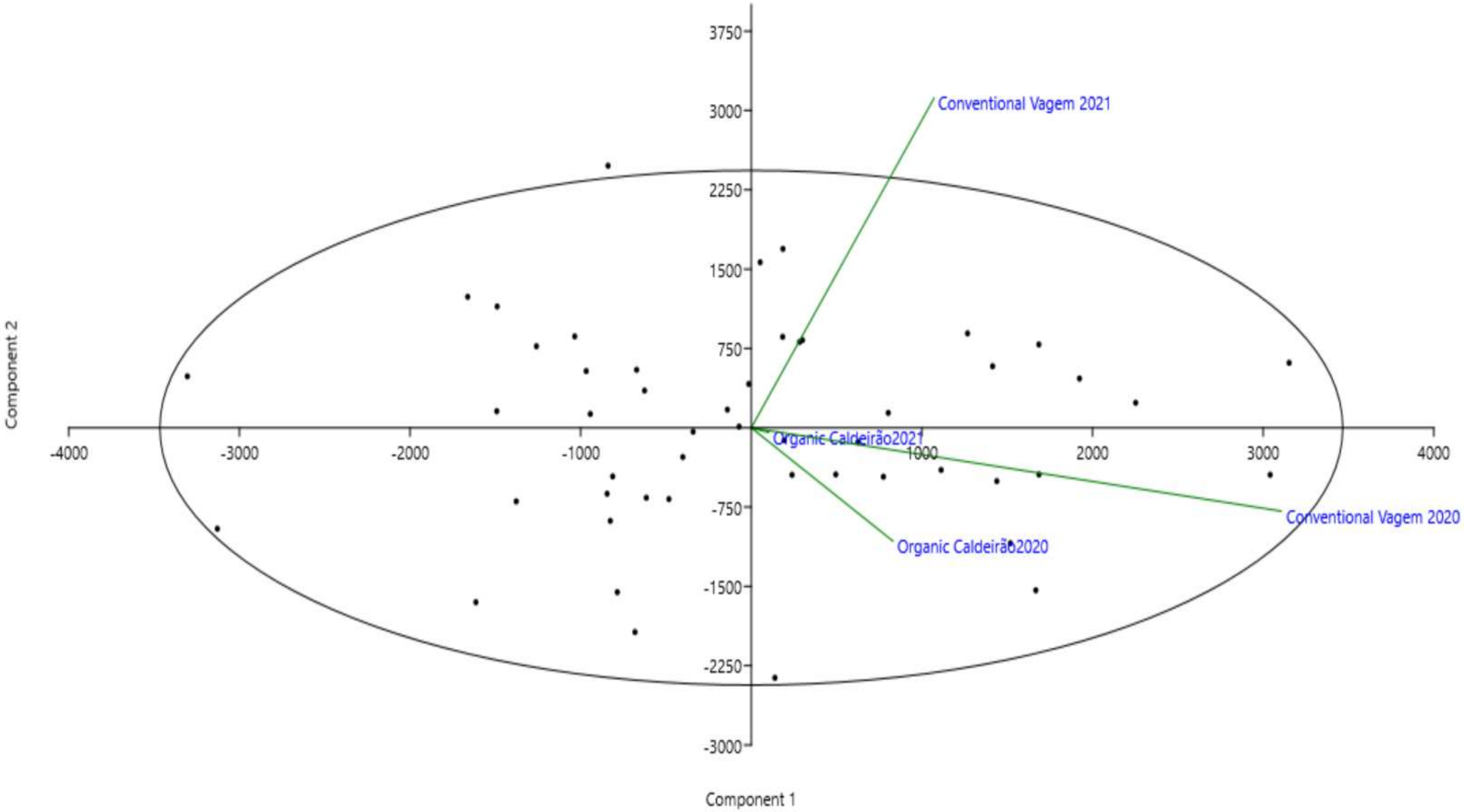


Figure 20- The results of the Biplot Analysis concerning genotypes and environments average scores of Grain yield in the Organic vs Conventional trial in 2020 and 2021 (Components 1 and 2 explain 53,7 % and 26,46 %. of the variance detected, respectably)

Figure 19 shows all the results of the maize populations and Figure 20 connects them (dots) to the biplot analysis showing the results of the vectors and environments. A closer look at Figure 20 shows a positive correlation between the grain yield data of Organic Caldeirão 2020, Conventional Vagem 2020 and Organic Caldeirão 2021, since the vector line shown has an acute angle between the environments. The Conventional Vagem 2021 environment showed no correlation, indicated by the straight angle between the vectors of the other environments.

Looking closely at the two graphs (Figures 19 and 20), we can see which maize populations (clusters) are better suited to organic environments because of their proximity to the environmental score vector. For organic environments where the vectors are strongly connected, we can select: 2519 - (Cald 19), 2448 - (Cald 19), 2494 - (Cald 19), 2501 - (Cald 19), 2504 - (Cald 19), 2517 - (Cald 19), Bulk Azores 2 - (Cald 19), and VA 19 - (Reg Lou 19).

2.8.4- Correlation between phenological parameters and Grain yield

Data from the Organic vs. Conventional trial (days to anthesis, days to silking, grain yield, and HUNTERS descriptor) were correlated using Pearson correlation (Table 21), and it was possible to identify the existence of factors with different significant positive correlations with grain yield in (H) plant height, (H1E) height of first ear, and (U) uniformity.

Table 21- Pearson's correlation with the data obtained from the characterization of plants and Grain Yield. Asterisk indicates statistical correlations (*: P<0.05; **: P<0.01; ***: P<0.001)

	Grain yield	Mi	Fi	Mf	Ff	H	H1E	U	N	T	E	%R	%S
Grain yield	--												
Mi	-,227**	--											
Fi	-,314**	,852**	--										
Mf	-,390**	,508**	,385**	--									
Ff	-,407**	,756**	,843**	,625**	--								
H	,468**	-,123**	-,144**	-0,046	-,136**	--							
H1E	,460**	-0,041	-0,048	-,122**	-,084*	,914**	--						
U	,329**	-,134**	-,206**	-,107**	-,228**	,381**	,355**	--					
N	-,334**	,167**	,184**	,247**	,235**	-,291**	-,305**	-,278**	--				
T	0,043	-,076*	-,075*	,168**	0,039	,164**	,129**	,126**	0,016	--			
E	0,032	0,064	,097**	,181**	,150**	,288**	,345**	-0,015	-0,045	0,064	--		
%R	-,334**	,104**	,146**	,409**	,264**	0,007	-0,061	-,082*	,210**	,137**	,077*	--	
%S	-,245**	0,038	0,046	,574**	,254**	,097**	0,007	-0,058	,076*	,185**	,231**	,108**	--

The positive correlations between grain yields indicate that larger plants with higher ear placement and uniformity were more productive. A detailed look at Table 21 also shows negative correlations with grain yield by days to anthesis (Mi), days to silking (Fi), days to-end-of anthesis (Mf), days to-end-silking (Ff), leaf angle (N), root lodging (R), and stalk lodging (S). The former correlations are consistent with the highly correlated values between days to-end-anthesis (Mf) on the one hand and root lodging (R) and stalk lodging (S) on the other, suggesting that populations with long cycles tend to be less productive. This can be explained by late sowing and the resulting higher exposure to adverse weather conditions in the last months of the maize cycle, which favoured yield loss of populations with longer cycles.

The negative correlation of grain yield with leaf angle (N) can be explained by the low stand (plant density) that allowed the maize plants to spread and express this trait on plots that were consequently less productive.

Overall, the results agreed to some extent with those obtained by other authors (Aziz et al., 1998; Bocanski et al., 2009). Grain yield and plant height had high, positive connections, while first ear height and grain yield had moderate correlations.

According to data collected in 2020 (Annexes V, VI, VII, and VIII) from biometric ear characterization and correlated with grain yield values using Pearson's correlation (Table 22), it was possible to identify the existence of significant and important correlations, which were signaled with a color gradient corresponding to the degree of correlation obtained between parameters tested and Grain Yield, such as:

- Length of the ear; L = 0.718
- Values of the ear diameters; ED1= 0.544; ED3= 0.693; ED2= 0.568 and ED4= 0.492
- Ear weight; EW=0.82.
- Cob weight; EW= 0.694.
- Number of rows; NC=0.78
- Values of the cob diameters; DC2=0.292 and DC4=0.388.

Grain yield had the strongest correlation with ear weight, followed by the number of rows, length of the ear, and cob weight. Our findings are in accordance with the findings of other researchers (Bocanski et al., 2009; Duarte Pintado, 2019), which had the highest phenotypic coefficients of correlation between grain yield on one hand and cob weight, ear weight, or ear length on the other. Furthermore, only Ear convulsion (CV=0.269) showed Medium negative relationships (Bocanski et al., 2009; Duarte Pintado, 2019)

Table 22- Pearson's correlation with the data obtained from the characterization of ears according to the HUNTERS descriptor and Grain Yield (Yld15)

	Yld15	L	ED1	ED3	ED2	ED4	R1	R2	Fa	D/I	TR	CV	F/D	KC	EW	CW	KW15	KD	SW	HR	NC	DC1	DC3	DC2	DC4	M1	M2	Rq1	Rq2
L	0,718																												
ED1	0,544	,490**																											
ED3	0,693	,639**	,833**																										
ED2	0,568	,489**	,852**	,926**																									
ED4	0,492	,398**	,665**	,771**	,806**																								
R1	0,126	0,054	,378**	,273**	,340**	,182**																							
R2	0,131	0,065	,390**	,278**	,360**	,196**	,961**																						
Fa	-0,121	-,204**	0,096	-0,006	,130*	0,045	,282**	,289**																					
D/I	-0,011	-0,046	0,004	0,013	-0,011	-0,005	-0,012	-0,009	-0,053																				
TR	-0,036	-,155**	-0,002	0,062	0,082	0,082	,123*	,103*	,140**	0,038																			
CV	-0,269	-,405**	0,045	-,102*	0,021	0,019	,219**	,199**	,268**	0,005	,278**																		
F/D	0,214	,246**	,215**	,318**	,275**	,252**	-,369**	-,366**	-,138*	-0,045	-,126*	-,283**																	
KC	-0,016	,132*	0,005	0,024	0,006	-0,013	0,03	0,048	-0,059	-0,063	-0,004	-0,068	-0,046																
EW	0,82	,842**	,680**	,847**	,721**	,593**	0,097	,110*	-,124*	-0,03	-0,075	-,314**	,365**	0,015															
CW	0,694	,790**	,721**	,856**	,771**	,625**	,128*	,142**	-0,095	-0,049	-0,041	-,268**	,308**	0,074	,908**														
KW15	0,824	,831**	,656**	,827**	,700**	,579**	0,082	0,096	-,121*	-0,025	-0,07	-,322**	,374**	-0,005	,985**	,867**													
KD	-0,008	0,01	0,081	0,067	,119*	0,055	,195**	,180**	0,088	0,001	0,034	0,081	-,199**	-0,001	0,018	0,042	0,02												
SW	0,517	,501**	,416**	,605**	,558**	,508**	-,413**	-,419**	-,188**	-0,006	0,018	-,250**	,512**	-0,062	,662**	,625**	,661**	-0,077											
HR	0,343	,435**	,307**	,323**	,195**	,149**	,162**	,145**	-,120*	0,017	-,192**	-,183**	,142**	-0,082	,473**	,362**	,441**	-0,052	,162**										
NC	0,78	,886**	,482**	,644**	,462**	,391**	0,036	0,051	-,261**	-0,033	-,133*	-,422**	,262**	0,025	,880**	,758**	,880**	0,002	,499**	,474**									
DC1	0,117	,104*	,166**	,168**	,164**	,122*	,121*	,116*	0,048	-0,011	0,058	0,044	0,028	0,007	,131*	,169**	,123*	0,044	0,058	0,016	0,087								
DC3	0,103	,109*	,133*	,144**	,117*	0,08	0,045	0,046	0,083	-0,009	,105*	0,066	0,049	0,001	,138*	,146**	,133*	0,016	0,086	0,06	0,093	0,04							
DC2	0,292	,320**	,626**	,566**	,611**	,425**	,586**	,604**	,269**	-0,055	0,079	,122*	-0,051	0,022	,441**	,538**	,405**	,149**	0,049	,281**	,261**	,158**	,170**						
DC4	0,388	,421**	,613**	,611**	,614**	,451**	,467**	,483**	,211**	-0,069	0,033	0,03	0,026	0,045	,540**	,632**	,501**	,111*	,159**	,286**	,364**	,165**	,198**	,826**					
M1	0,141	,104*	,488**	,367**	,468**	,309**	,688**	,706**	,313**	-0,038	,131*	,267**	-,231**	0,009	,181**	,258**	,163**	,243**	-,197**	,102*	0,054	,131*	,103*	,650**	,530**				
M2	0,014	0,001	,152**	,150**	,192**	,145**	,173**	,181**	,130*	-0,018	0,023	-0,002	0,031	-0,029	0,049	0,071	0,045	0,048	-0,008	0,026	0,012	0,032	0,018	,167**	,163**	,239**			
Rq1	-0,075	-0,066	0,018	0,003	0,017	-0,004	,102*	,104*	0,035	-0,01	0,001	0,095	0,002	0,074	-0,075	-0,053	-0,063	0,018	-,099*	0,02	-0,059	0,004	0,003	0,045	0,016	,111*	0,024		
Rq2	0,117	,122*	,334**	,278**	,316**	,227**	,454**	,458**	,416**	-0,024	,107*	,186**	-,112*	-0,006	,185**	,257**	,161**	,137*	-,113*	,181**	0,05	,125*	,131*	,532**	,525**	,708**	,211**	,154**	
CC	0,049	0,074	0,03	0,003	0,005	0,02	,195**	,205**	-0,001	-,105*	-0,031	-0,006	-0,038	-0,083	0,037	0,028	0,046	0,057	-,124*	0,09	0,077	0,042	0,054	0,087	,108*	,143**	0,078	-0,06	0,084

Color gradient corresponding to the degree of correlation obtained between parameters													
-0,3	-0,2	-0,1	0,0	0,1	0,2	0,3	0,4	0,5	0,6	0,7	0,8	0,9	1,0

Chapter 3- On-Farm PPB trial

3.1- Trial Location

The On-Farm PPB Trial were managed at Alvarenga, Lousada, Portugal (41.29412380145764; -8.26727628707886; 286 m elevation) and Macieira de Lixa, Felgueiras, Portugal (41.345100, -8.169200; 406 m elevation), located in the heart of a triangle classified by UNESCO as a World Heritage Site (Figure 21), including Porto, Guimarães and the Douro Valley (Ader-Sousa, 2018).

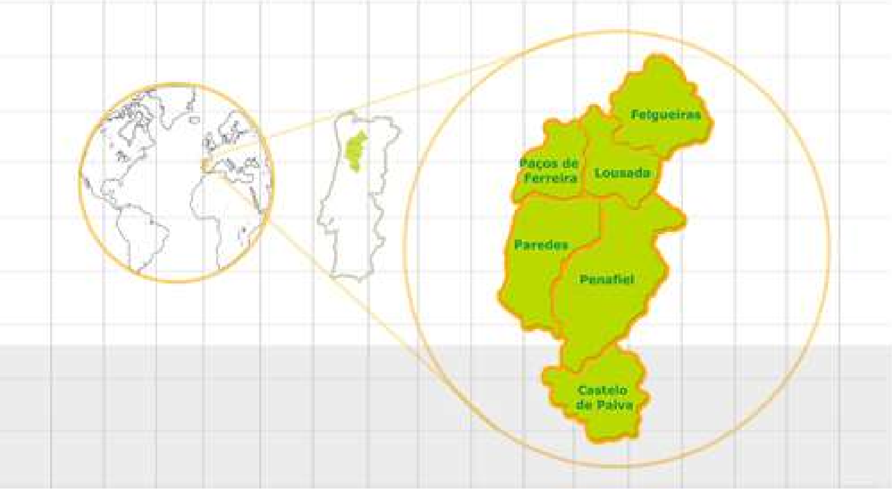


Figure 21- Location and municipalities of Vale do Sousa, Portugal ***(Source: ADERSOUSA)***

3.2- Trial edaphoclimatic conditions

The climate in Vale do Sousa is temperate, with four distinct seasons and more rainfall in winter. The climate of Macieira de Lixa and Lousada, according to Koppen and Geiser's classification, is Csc, a warm climate with cool summers (Climate, 2021; Kottek et al., 2006). Macieira de Lixa and Lousada show an average annual temperature of 14,9°C and an average month temperature of 17,2°C in May, 18,6°C in June, 21,7°C in July, 22,1°C in August, and 20,2°C in September (AccuWeather, 2020; IPMA, 2021).

When the trials were implanted, soil samples were obtained to measure physical and nutritional soil properties; the results are shown in Table 23.

Table 23- Soil analysis (On-Farm PPB) in 2020 and 2021 (Annex II-IV)

On-Farm PPB trial					
Parameter	**Unit**	**Macieira 2020**	**Lousada 2020**	**Macieira 2021**	**Lousada 2021**
Soil texture		Medium	Medium	Medium	Medium
Soil <2mm	**%**	100	81,46	95,03	82,04
Org. Mat	**%**	2,8 (medium)	4,3 (medium)	2,8 (medium)	3,5 (medium)
pH	**H2O**	5,3 (acid)	5,9 (few acid)	5,7 (few acid)	6 (few acid)
P_2O_5	**mg/kg:**	8 (very low)	117 (high)	19 (very low)	96 (medium)
k_2O	**mg/kg:**	101(very high)	421 (very high)	194 (very high)	280 (very high)

3.3- Germplasm used

The germplasm used in the 2020 and 2021 trials (Table 24) included:
-8 accessions were derived from 4 OPPs obtained from the participatory plant breeding program ("VASO") in Sousa Valley that has been active in the northern part of Portugal since 1984:

- ‘Pigarro’ is a white flint type FAO 300 cycle, with high kernel-row numbers (18 to 28), selected from a traditional Portuguese landrace (3 accessions: Pg 19 (Lou 19); Pg 18 (Cald 18) and Pg 19 (Cald 19) - subjected to stratified mass selection in ESAC in 2018 and 2019).
- ‘Verdeal´ is a white flint type FAO 300 cycle, selected from a traditional Portuguese landrace (3 accessions: VA 17 (Seq Lou 17), VA 19 (Reg Lou 19), VA 19 (Sequeiro Lou 19) - subjected to stratified mass selection on-farm in Lousada, Portugal in different irrigation systems in 2017 and 2019).
- ‘Fandango’ is a yellow dent type FAO 600 cycle synthetic composite, selected from the intercrossing of 77 yellow, elite inbred lines (dent and flint; 20% Portuguese and 80% American germplasm), with the big kernel-row number and large ear size (1 accession: FN-2014 (Lou 19) - subjected to stratified mass selection on-farm in Lousada, Portugal in 2019).
- ‘Amiúdo’ is a yellow flint early population (FAO 200) adapted to poor soils (1 accession: Am C397 (Lou 19) - subjected to stratified mass selection on-farm in Lousada, Portugal in 2019).

-2 Composite Cross Populations (CCP) obtained using a polycross method based on 'Nutica' experience (Mendes-Moreira et al., 2009): 'SinPre' (Sintético Precoce) obtained through the crossing of 12 maize populations (10 Portuguese landraces and 2 American populations) subjected to on-farm stratified mass selection under PPB ("VASO") and multiplied in 2019 at ESAC. ‘BulkAzores2’ were obtained in 2019 through the crossing of 40 Azorean OPPs and subjected to mass selection at ESAC and on-farm in 2019.

Table 24 - The origin, selection site, and selection Agro-system of the germplasm used in 2020 and 2021 (On-Farm PPB trial).

On-Farm PPB trial (germplasm used)			
Genotype	**Origin**	**Selection Site**	**Selection Agro-system**
Amc397- (Lou 19)	Vale do Sousa Portugal	Lousada, Portugal	Low-input Conventional
Fn 2014- (Lou 19)	Vale do Sousa Portugal	Lousada, Portugal	Low-input Conventional
Pg 18- (Cald 18)	Vale do Sousa Portugal	Coimbra, Portugal	Low-input Organic
Pg 19- (Cald 19)	Vale do Sousa Portugal	Coimbra, Portugal	Low-input Organic
Pg 19 - (Lou 19)	Vale do Sousa Portugal	Lousada, Portugal	Low-input Conventional
SinPre- (Cald 19) CCP	Vale do Sousa Portugal	Coimbra, Portugal	Low-input Organic
VA 17 - (Seq Lou 17)	Vale do Sousa Portugal	Lousada, Portugal	Agroecology Conventional (drought)
VA 19 - (Reg Lou 19)	Vale do Sousa Portugal	Lousada, Portugal	Agroecology Conventional (irrigated)
VA 19 - (Seq Lou 19)	Vale do Sousa Portugal	Lousada, Portugal	Agroecology Conventional (drought)
Bulk-Azores 2- (Cald 19) CCP	Coimbra, Portugal	Coimbra, Portugal	Low-input Organic

Seeds were dried and kept in cold storage (4 ◦C) until their use.

3.4- Experimental design

The accessions were evaluated in a randomized complete block (RCBD) design with 60 000 stands, in plots of two lines with 6.4 lengths and 0,8 interrow distance (Figure 22).

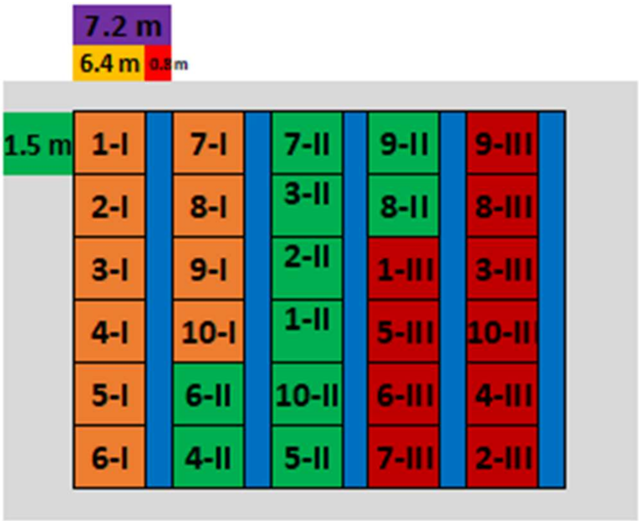


Figure 22- On-farm PPB trial design (2020 and 2021).

3.5- Agronomic practices

Soil fertilization was done in all environments the same way in both years: 1 month before sowing with approximately 15 Mg of animal manure, which represents 75 kg/ha of N, 30 kg/ha of P_2O_5, and 150 kg/ha of k_2O (Direção Geral de Agricultura e Pescas de Lisboa e Vale do Tejo, 2012). Tables 25 and 26 detail the field activities at each trial site over the course of two years (2020-2021).

Table 25-Field activities during the year 2020

Site	Ploughing	Seedbed Preparation	Design of the sowing Lines	Sowing	Weed Control	Harvest
Macieira de Lixa	18/05/2020	30/05/2020	30/05/2020	30/05/2020	Mechanic	19/10/2020
Lousada	15/05/2020	25/05/2020	25/05/2020	25/05/2020	Mechanic	08/10/2020

Table 26- Field activities during the year 2021

Site	Ploughing	Seedbed Preparation	Design of the sowing Lines	Sowing	Weed Control	Harvest
Macieira de Lixa	15/05/2021	25/05/2021	01/06/2021	01/06/2021	Mechanic	25/10/2021
Lousada	21/05/2021	24/05/2021	28/05/2021	28/05/2021	Mechanic	11/10/2021

3.6- Agronomic characterization and on-field evaluation

Data from the morphologic parameters of the plant, the biometric parameters associated with the ear, and the grain yield of the 10 maize populations were collected during the maize crop cycle, as mentioned in section 2.6 of this document.

Furthermore, six genotypes were chosen from the tested populations: three genotypes of 'Verdeal' (VA) and three genotypes of 'Pigarro' (Pg) that represent different years of mass selection in different environments and gastronomic potential for maize bread. In two years, these six populations were tested in three environments (Macieira de Lixa, Lousada, and the Organic Caldeirão in Coimbra). This framework enabled grain yield comparisons (PCA analysis) between environments of maize populations with gastronomic potential (Maize Bread) and forecasting the best selection environments suitable for use in PPB programs adapted to Organic and low-input farming.

The trial was also planned to allow for additional empirical analysis by Sousa Valley farmers and stakeholders. Within this context, on-field evaluations were carried out in the Macieira de Lixa field on the 15th of October 2020 (Harvest period), as part of a survey (Annex XI). Participants rated the ten maize populations tested on five important morphological traits: plant height, uniformity, ear height, ear size, and grain type. The scale used was hedonic, with nine points ranging from 9 (I liked it a lot) to 1 (I disliked it a lot). The scores of the three plots (repetitions) of each maize population were added together to select the three best maize populations in each trait (Figure 22).

Ultimately, participants were asked to identify the maize population with the best agronomic behavior suitable for gastronomic (Maize bread) and animal feed purposes (Silage).

This initiative was carried out as part of the European project LIVESEED and was funded by ADERSOUSA. Members of local municipal associations that support farmers, such as Cooperativa de Lousada, Cooperativa de Penafiel, Cooperativa de Felgueiras, and farmers from the Sousa Valley region, were encouraged to participate.

3.7- Statistical methods

To generate descriptive statistics and statistically significant differences, SPSS (Statistical Package for Social) Package 6, version 26 was used. First, morphological attributes and grain yield data were examined using ANOVA (one-way, two-way) to discover statistical differences based on environment, genotype, and year. The Tuckey test was then employed in homogenous subsets to differentiate means within each genotype. The differences were statistically significant at $P<0.05$. A simple rank approach was used to examine overall yield rank behavior in diverse environments, as well as to further assess the evolution of yield behavior and select viable cultivars for each environment.

Furthermore, Principal Component Analysis (PCA) and biplot analysis were used to classify and discover genotypes that are more productive in diverse contexts, as well as the optimum selection environments, using the software Past 4.03 and GGE biplot (analysis of the 6 maize populations with maize bread aptitude). A Pearson correlation was also performed using SPSS (Statistical Package for Social) Package 6, version 26 to assess the statistical relationships between HUNTERS descriptors, grain yield, and ear parameters.

Lastly, the on-field evaluation scores of the five morphologic characteristics were subjected to simple rank to determine the three best scores in each trait from the farmer's/perspective. stakeholder's

3.8- Results and Discussion

3.8.1- HUNTERS descriptor

The values for plant heights (Figure 23) ranged from 144.2 cm (AmC397- Lou 19) to 329.1 cm (Fn 2014 - Cald 19), while the values for first ear insertion heights (Figure 24) ranged from 76 cm (AmC397- Lou 19) to 219.6 cm (Fn 2014 - Cald 19).
A closer examination of the genotypes' plant height (H) and first ear height (H1E) values reveals significant differences (Table 27). However, there were no significant differences in Plant height (H) based on the environment or the year of the trial.
The maize populations studied exhibited a consistent height behavior.

*Table 27- - One-way ANOVA results on plant height (H) and first ear height (H1E) measurements. The asterisk * indicates statistical differences between the genotypes (*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$)*

			Sum of Squares	df	Mean Square	F	Sig.
H * Genotype	**Between Groups**	**(Combined)**	24180,401	9	2686,711	3,342	,001***
	Within Groups			109	803,917		
	Total			118			
H1E * Genotype	**Between Groups**	**(Combined)**	19006,001	9	2111,778	4,174	,000***
	Within Groups			109	505,978		
	Total			118			

Following that, the post hoc test is performed. Tuckey found statistically significant differences between genotypes and divided them into subsets (SUBSET-displayed next to the genotype name), as shown in Figures 23 and 24.
Fn 2014 – Cald 19 was the significantly taller maize population tested in 2014, while AmC397- Lou 19 was the significantly smaller maize population tested. The findings are strongly related to the longer cycle (FAO 600 cycle) of Fn 2014 – (Cald 19) and the shorter cycle (FAO 200 cycle) of AmC397- (Lou 19).
According to first ear height values, Fn 2014- (Cald 19) has significantly higher values, while AmC397- (Lou 19) and Sinpre- (Cald 19) have significantly lower values. Sinpre- (Cald 19) is a CCP (Composite Cross Population) created by breeders later than the other maize populations tested, and unlike the other Portuguese maize populations tested, the first plant selection was performed in the field. Since their original conception, the best ears to use as next year seed have been selected in the ground while the maize was drying after harvest; this selection method did not take into account any plant morphologic characteristics such as first ear eight.

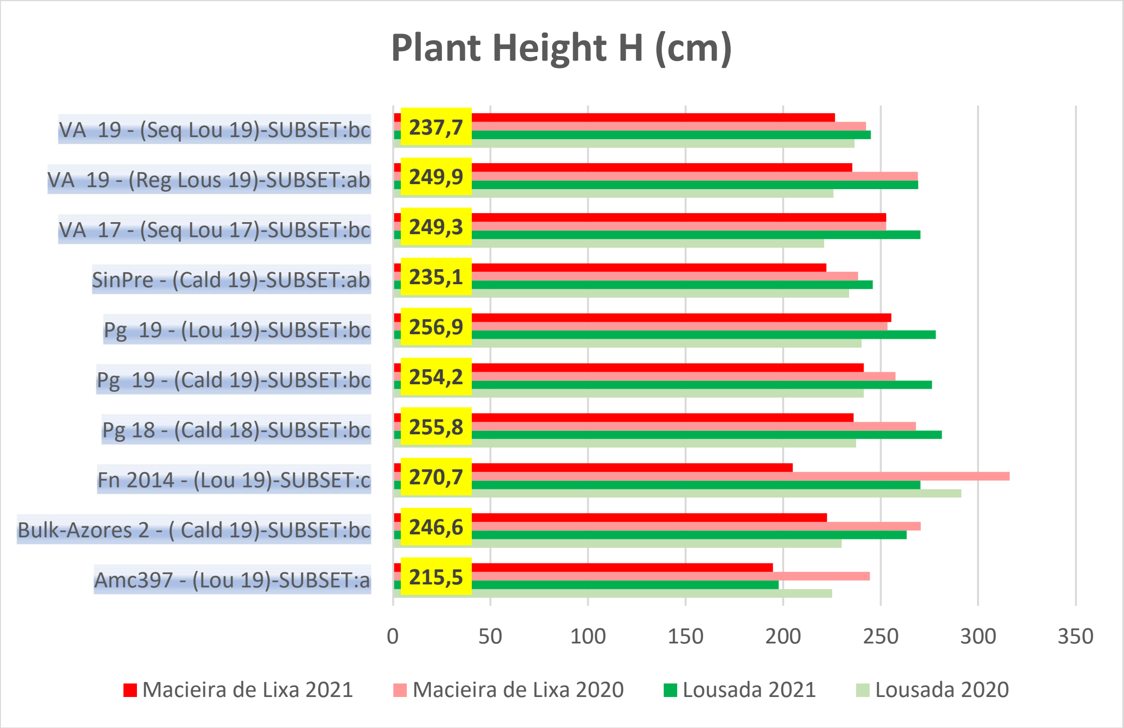


Figure 23- Plant height (H) average values of the genotypes by environment and year (bars) and Plant height (H) overall average values of the trial (values). SUBSET- represent the homogeneous subsets (Tukey's multiple comparisons test) with different significance; a=0,299, b=0,322, c=0,198)

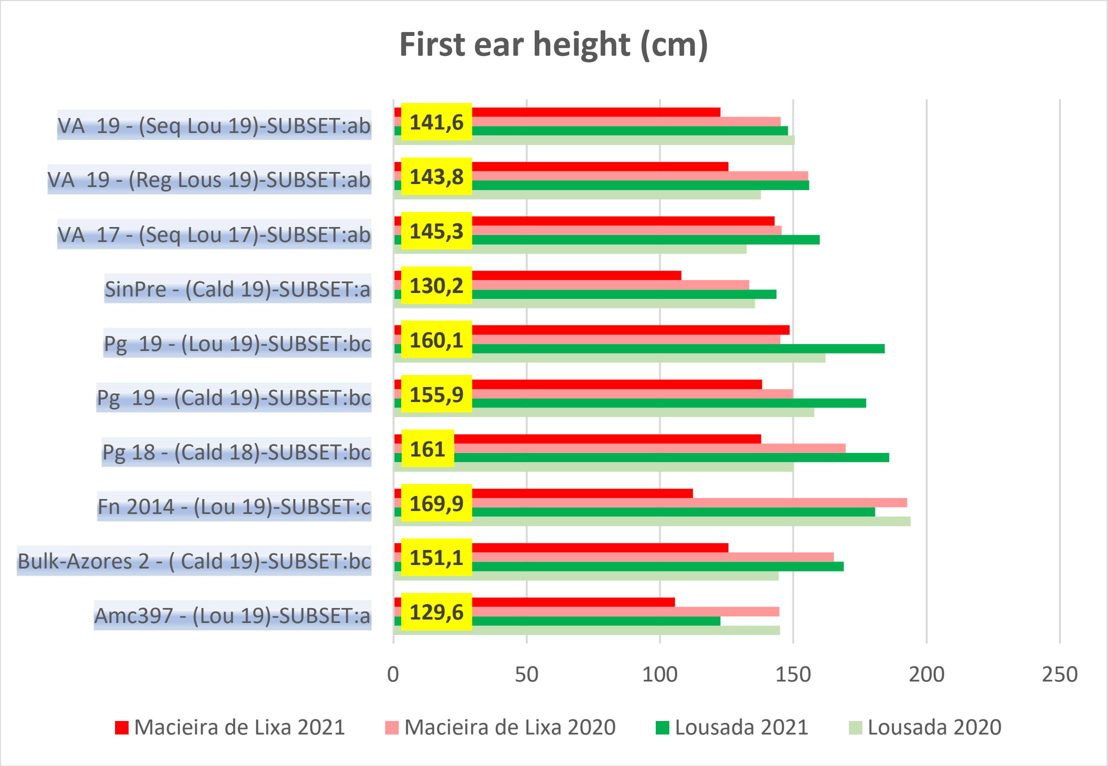


Figure 24- - First ear height (H) average values of the genotypes by environment and year (bars) and First ear height (H) overall average values of the trial (values). SUBSET- represent the homogeneous subsets (Tukey's multiple comparisons test) with different significance; a=0,069, b=0,066, c=0,09)

The average genotype values for the uniformity parameter (U) (Table 28) show that all maize populations achieve values between 3 and 3.99. These uniformity values indicate a high level of genetic diversity within populations, which is beneficial for plant adaptation and breeding (Costa-Rodrigues, 1971; Vaz Patto et al., 2008). However, no significant differences were discovered between the environments or over the course of the two-year trial.

According to data obtained on the leaf angle (N) (Table 28), the average genotype value ranged between 4.5 (VA 19 - (Sequeiro Lou 19)) and 5.8 (Amc397 - (Lou 19)). The higher N values imply that the maize populations studied were bred in polyculture systems with low sowing densities (Mendes-Moreira et al., 2017; Moreira, 2006).

The study of maize populations tassel (T) (Table 28) shows that they are huge and highly branching in general, with no notable variations across genotypes. ANOVA analysis, on the other hand, reveals a significantly higher Tassel score in 2021.

The placement or the insertion of the first ear (E) values (Table 28), indicates that most maize populations tested have ear placement above the middle of the plant. Manual harvesting can be a challenge in maize populations with E values above 6 (30%).

Table 28- - Averages referred to the HUNTERS descriptors (Uniformity, Leaf Angle, Tassel and Ear placement), used to characterize the maize genotypes of the On-Farm PPB trial

Genotype	U	N	T	E
Amc397 - (Lou 19)	3,1 ±0,9	5,8 ±0,8	5,4 ±1,4	5,6 ±0,7
Bulk-Azores 2 - (Cald 19)	3,6 ±0,7	5,6 ±0,5	5,9 ±1,2	5,8 ±0,7
Fn 2014 - (Lou 19)	3,2 ±0,9	5,6 ±0,7	5,9 ±1,2	6 ±0,9
Pg 18 - (Cald 18)	3,3 ±0,6	5,7 ±0,8	6,3 ±1,4	6,1 ±0,7
Pg 19 - (Cald 19)	3,1 ±0,7	5,4 ±1,1	6,1 ±1,2	6 ±0,6
Pg 19 - (Lou 19)	3,6 ±0,7	5,6 ±0,8	6,1 ±1,4	5,9 ±0,3
SinPre - (Cald 19)	3,2 ±0,8	5,7 ±0,7	5,3 ±1	5,7 ±0,5
VA 17 - (Seq Lou 17)	3,2 ±0,7	5 ±0,7	5,5 ±1,3	5,3 ±0,5
VA 19 - (Reg Lou 19)	3,6 ±0,7	4,5 ±0,7	5,7 ±0,8	5,6 ±0,7
VA 19 - (Seq Lou 19)	3,1 ±0,7	4,5 ±1,1	5,3 ±0,9	5,5 ±0,5

Root lodging (R) plot values (Figure 25) ranged from 0 (several genotypes) to 86.04 % (Fn- 2014- (Lou 19), and Stalk lodging (S) plot values (Figure 26) ranged from 0 (several genotypes) to 57.14 % (Bulk-Azores2- (Cald 19).

There were no significant changes in root lodging (R) values between maize populations, environment, or trial year. Stalk lodging (S) values, on the other hand, revealed significant differences between maize populations, environments, and trial year (Tables 29 and 30).

Macieira de Lixa had significantly higher average values (14.84 %) than Lousada (6.58 %). In terms of the trial year, 2020 (12.63 %) had significantly higher values than 2021 (10.63 %). This may be related to the Macieira de Lixa field's higher wind exposure.

However, studies on maize stalk morphology have revealed that maize plants with a higher ear position and center of gravity are more likely to lodge (Xue et al., 2020), and several of the Portuguese maize populations in the study exhibit these characteristics (Figures 25 and 26).

*Table 29- - One-way ANOVA results about Stalk lodging (S) values. The asterisk * indicates statistical differences between the environments (*: P < 0.05; **: P < 0.01; ***: P < 0.001)*

			Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
%S * Environment	**Between Groups**	**(Combined)**	2033,920	1	2033,920	32,557	,000***
	Within Groups		7309,182	117	62,472		
	Total		9343,102	118			

*Table 30- - One-way ANOVA results about Stalk lodging (S) values. The asterisk * indicates statistical differences between the trial year (*: P < 0.05; **: P < 0.01; ***: P < 0.001)*

			Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
%S * Year	**Between Groups**	**(Combined)**	442,852	1	442,852	5,822	,017**:
	Within Groups		8900,250	117	76,071		
	Total		9343,102	118			

Afterward, the post hoc test Tuckey identified statistically significant differences within the genotypes regarding Stalk lodging (S) and divided were into sub-sets (SUBSET- displayed next to the genotype name), as shown in the graph representation in Figure 26.

The highlights go to VA 17- (Seq Lou 17) with significantly lower values in Stalk lodging (S), and Bulk-Azores2- (Cald 19) with significantly higher values.

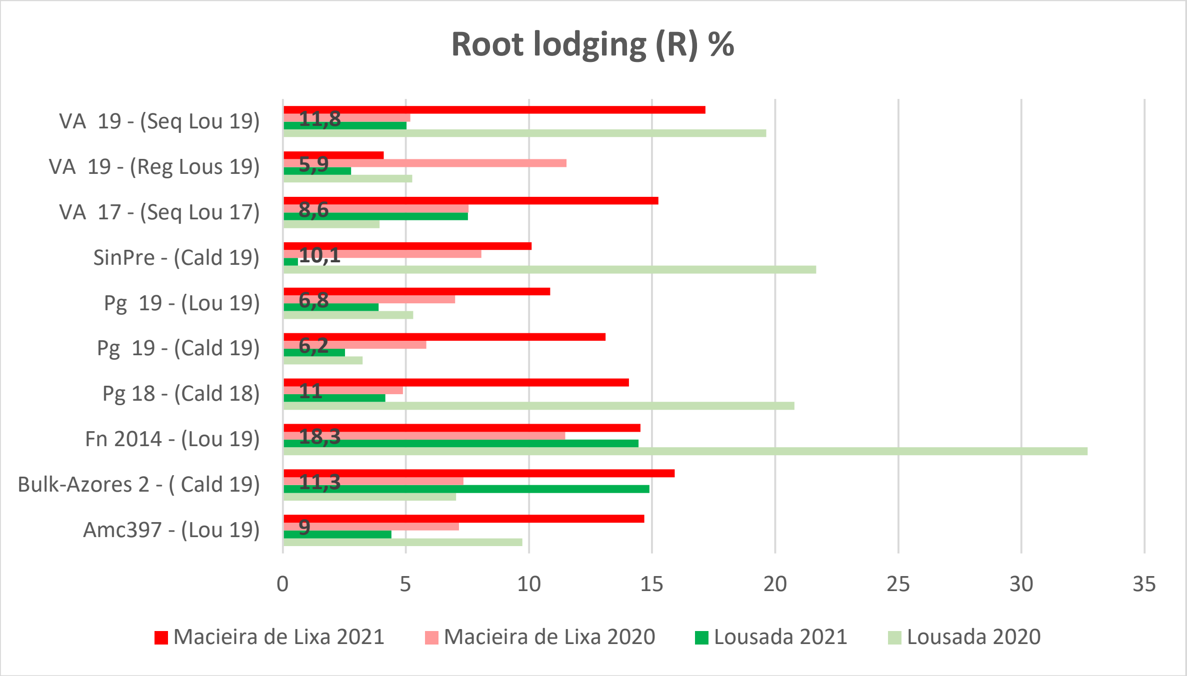


*Figure 25 - Root lodging (R) average values of the genotypes by environment and year (bars) and Root lodging (R) overall average values of the On-farm PPB trial (**values**).*

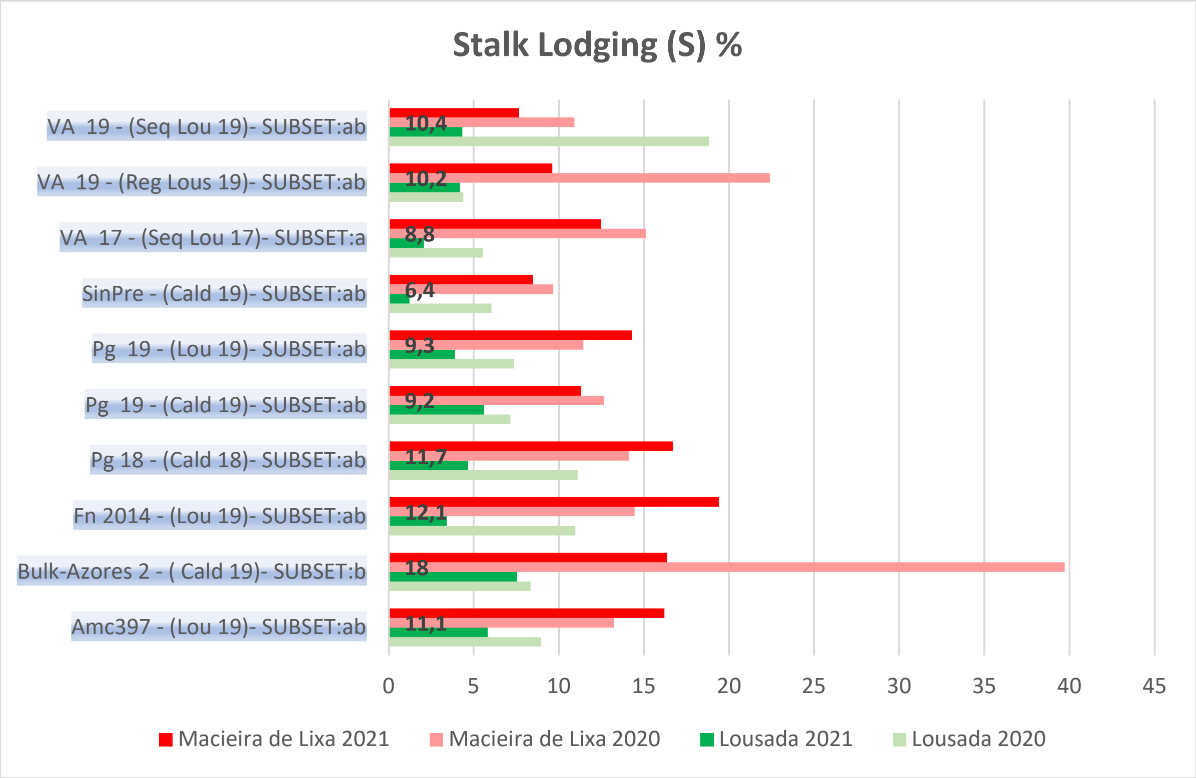


*Figure 26- Stalk lodging (S) average values of the genotypes by environment and year (bars) and Root lodging (R) overall average values of the On-farm PPB trial (**values**). SUBSET- represent the homogeneous subsets (Tukey's multiple comparisons test) with different significance; a=0,058, b=0,053.*

3.8.2- Grain Yield evaluations

Table 31 shows the averages and standard deviations (SD) of grain yield data collected over two years. There were significant differences in grain yield average values between environments, genotypes, environments by year, and genotypes by year (Table 32). Grain yield averages ranged from 2 776.3 kg/ha (Amc397 - Lou 19) to 9 223.4 kg/ha (Fn 2014 - Lou 19), with Macieira de Lixa 2020 showing statistically significant higher values. Following that, the Tuckey post hoc test identified statistically significant differences within genotypes and divided them into sub-sets, as shown in Table 31.

Table 31- Grain yield data and SD from the two years (2020-2021) of the trial On-Farm PPB. (Letters represent the homogeneous subsets with different significance; a=0,085, b=0,075)

Genotype	Sub-sets	Lousada 2020		Macieira de Lixa 2020		Lousada 2021		Macieira de Lixa 2020	
		Yield (kg)	**SD (±)**	**Yield (kg)**	**SD (±)**	**Yield (kg)**	**SD (±)**	**Yield (kg)**	**SD (±)**
Amc397 - (Lou 19)	**b**	3623,6	1451,5	5590,8	720,4	3783,9	211,5	2776,3	1526,9
Bulk-Azores 2 - (Cald 19)	**a b**	4086,7	302,9	4091,3	861,7	5338,6	635,7	3726,1	1543,3
Fn 2014 - (Lou 19)	**a**	6949,3	2161,4	9223,4	1014,9	3670,3	868,0	3562,8	2952,8
Pg 18 - (Cald 18)	**a b**	3668,3	710,1	7231,9	1176,6	5705,7	1192,7	5799,4	783,3
Pg 19 - (Cald 19)	**a b**	4450,8	545,4	6667,3	3549,8	5835,5	836,5	6450,8	2147,2
Pg 19 - (Lou 19)	**a**	4139,8	689,8	7247,3	1639,0	6241,4	1392,9	6713,8	596,0
SinPre - (Cald 19)	**a b**	4085,0	1919,6	6628,8	1732,6	5214,3	1920,5	4406,6	1491,7
VA 17 - (Seq Lous 17)	**a b**	3635,3	882,2	5837,2	2091,8	5431,7	611,0	5338,1	1735,7
VA 19 - (Reg Lou 19)	**a**	6247,1	2127,0	7270,1	1951,8	5165,5	1618,9	5592,6	1315,8
VA 19 - (Seq Lou 19)	**a b**	4164,0	251,8	5521,4	2331,7	5122,1	302,9	5867,0	302,6

*Table 32- Tests of Between-Subjects Effects (Year, Environment, Genotype) in the two years. The asterisk * indicates statistical interaction (*: P<0.05; **: P<0.01; ***: P<0.001)*

Dependent Variable: Grain Yield					
Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Corrected Model	253706530,147[a]	39	6505295,645	3,037	,000
Intercepto	3372840983,788	1	3372840983,788	1574,849	,000
Environment	30427330,397	1	30427330,397	14,207	,000***
Genotype	63139148,377	9	7015460,931	3,276	,002***
Year	7260784,109	1	7260784,109	3,390	,069
Environment * Genotype	16838038,152	9	1870893,128	,874	,552
Environment * Year	38569181,601	1	38569181,601	18,009	,000***
Genotype * Year	88797200,259	9	9866355,584	4,607	,000***
Environment * Genotype * Year	8666863,979	9	962984,887	,450	,903
Error	169193678,451	79	2141692,132		
Total	3795239265,495	119			
Corrected Total	422900208,599	118			
a. R Squared = ,600 (Adjusted R Squared = ,402)					

The On-Farm PPB Trial demonstrates a captivating level of grain yield based on fertilization levels in both systems. A closer look at grain yield levels reveals a very different grain yield behavior of 'Fandango' - Fn 2014 - (Lou 19) between years (Figure 27), with higher production per plot in Macieira de Lixa in 2020 (11 538.9 kg/ha) and a significant decrease (6 972.4 kg/ha) in 2021. Despite this, Pg 19 (Lou 19) and VA 19 - (Reg Lou 19) had significantly higher averages (Table 31).

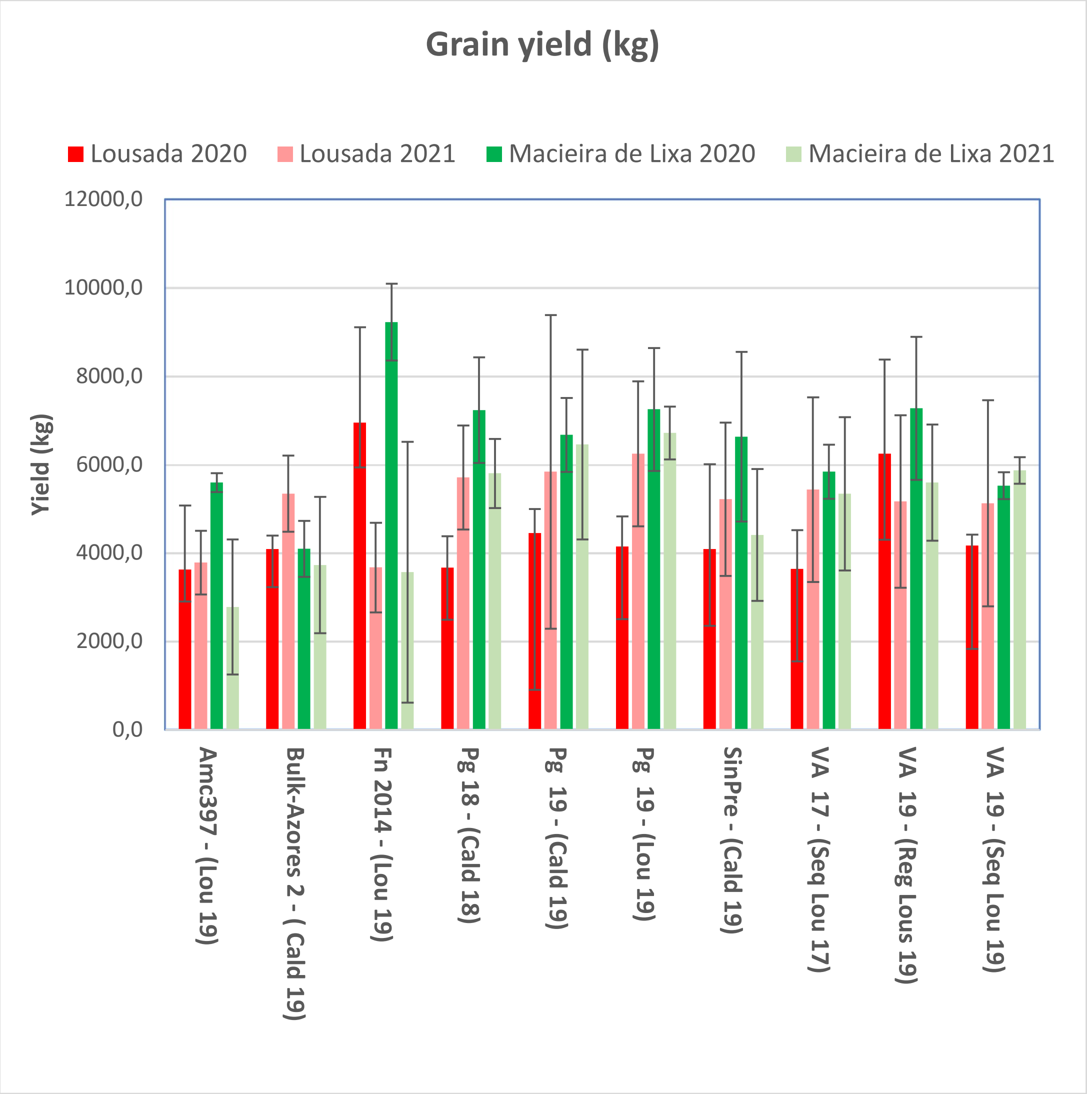


Figure 27 Grain yield average values and standard deviations of the genotypes by environment and year (On-Farm PPB trial)

Grain yield rankings (Table 32) show maize populations Pg 19 (Lou 19), Pg 19 (Cald 19), and VA 19 - (Reg Lou 19) as having the highest grain yields in both environments (data from two years). Pg 19 (Cald 19) in Macieira de Lixa had the highest overall grain yield average, and Pg 19 (Lou 19) in Lousada had the highest overall grain yield average. This single difference in grain yield ranking (top 3) between environments indicates that the environment of selection is important (Lousada), and confirms PPB's previous efforts to evaluate and select varieties for the farmers' system (Kovács & Pedersen, 2019).

Overall, the 'Pigarro' populations Pg 19 (Lou 19), Pg 19 (Cald 19), and Pg 18 (Cald 18) demonstrated grain rank yield stability and promising yield results in a variety of locations (Table 33). The less productive year (2021) of Fn 2014 - (Lou 19) had a greater impact on overall grain yield rank (5th). In both analyses, Amc397 – (Lou 19) had the poorest agronomic results (ANOVA, Grain yield rank).

Table 33- Overall rank and rank index of the On-Farm PPB trial ***(1- Macieira de Lixa 2020; 2- Macieira de Lixa 2021; 3- Lousada 2020; 4- Lousada 2021)***

Genotype	1	2	3	4	Rank ∑	Rank Macieira	Rank Lousada	Overall Rank
Pg 19 - (Lou 19)	5	1	3	1	10	2	1	1
Pg 19 - (Cald 19)	3	2	5	2	12	1	2	2
VA 19 - (Reg Lou 19)	2	7	2	5	16	3	3	3
Pg 18 - (Cald 18)	8	3	4	4	19	4	4	4
Fn 2014 - (Lou 19)	1	10	1	9	21	5	5	5
VA 19 - (Seq Lou 19)	4	8	9	3	24	7	6	6
VA 17 - (Seq Lou 17)	9	4	7	6	26	8	7	7
SinPre - (Cald 19)	7	6	6	7	26	9	8	8
BulkAzores2-(Cald 19)	6	5	10	8	29	6	9	9
Amc397 - (Lou 19)	10	9	8	10	37	10	10	10

Figures 28 and 29 show a PCA analysis of grain yield values from both years of the trial, as well as a biplot analysis. Figure 28 shows all of the maize population scores, and Figure 29 connects them (dots) to the biplot analysis, which shows the vectors and environments scores.

A more detailed look revealed two clusters.

- **Cluster 1**- 2 accessions of 'Verdeal': VA 19 (Seq Lou 19) and VA 17 (Seq Lous 17)
- **Cluster 2**- 3 accessions of 'Pigarro': Pg 19 (Lou 19); Pg 18 (Cald 18) and Pg 19 (Cald 19)

The first two clusters show two maize populations (Verdeal e Pigarro) in distinct selection years and locations.

The 'Verdeal cluster' (Cluster 1) includes the mass selections of 2017 and 2019 in a non - irrigated environment, whereas the remaining Verdeal genotype in the trial - VA 19 (Reg Lou 19) - was selected in an irrigated environment in 2019 and demonstrated greater grain production performance. We can assume that mass selection in irrigated conditions will be more effective for farmers in the Sousa Valley.

The 'Pigarro cluster' (Cluster 2) includes all three Pigarro (Pg) genotypes present in the trial with mass selections in different habitats and years. A closer look at Figure 29 reveals that Pg 19 (Lou 19) reached the highest score, followed by Pg 19 (Cald 19) and Pg 18 (Cald 18). The mass selection performed in the Sousa Valley location (Lousada) represented by maize population Pg 19 (Lou 19) was more effective in terms of grain yield than the mass selection performed in the low input organic field (Caldeirão) represented by Pg 19 (Cald 19) and Pg 18 (Cald 18). (Cald 18). Increases the significance of participatory breeding and pre-breeding operations such as cultivar testing in specific places. Furthermore, the development of mass selection with Pg 18 - (Cald 18) and Pg 19 - (Cald 19) representing two distinct years (2018 and 2019, respectively) was effective in terms of grain yield, with Pg 19 - (Cald 19) achieving a better score.

Figure 29 (biplot analysis) reveals a positive correlation between Grain yield data from Lousada 2020 and Macieira de Lixa 2020, as well as Lousada 2021 and Macieira de Lixa 2021, as the vector line depicted presents an acute angle between the two sets of environments. The straight angle between the vector lines from each year suggests that the edaphoclimatic conditions have a large impact in each year (Figure 29).

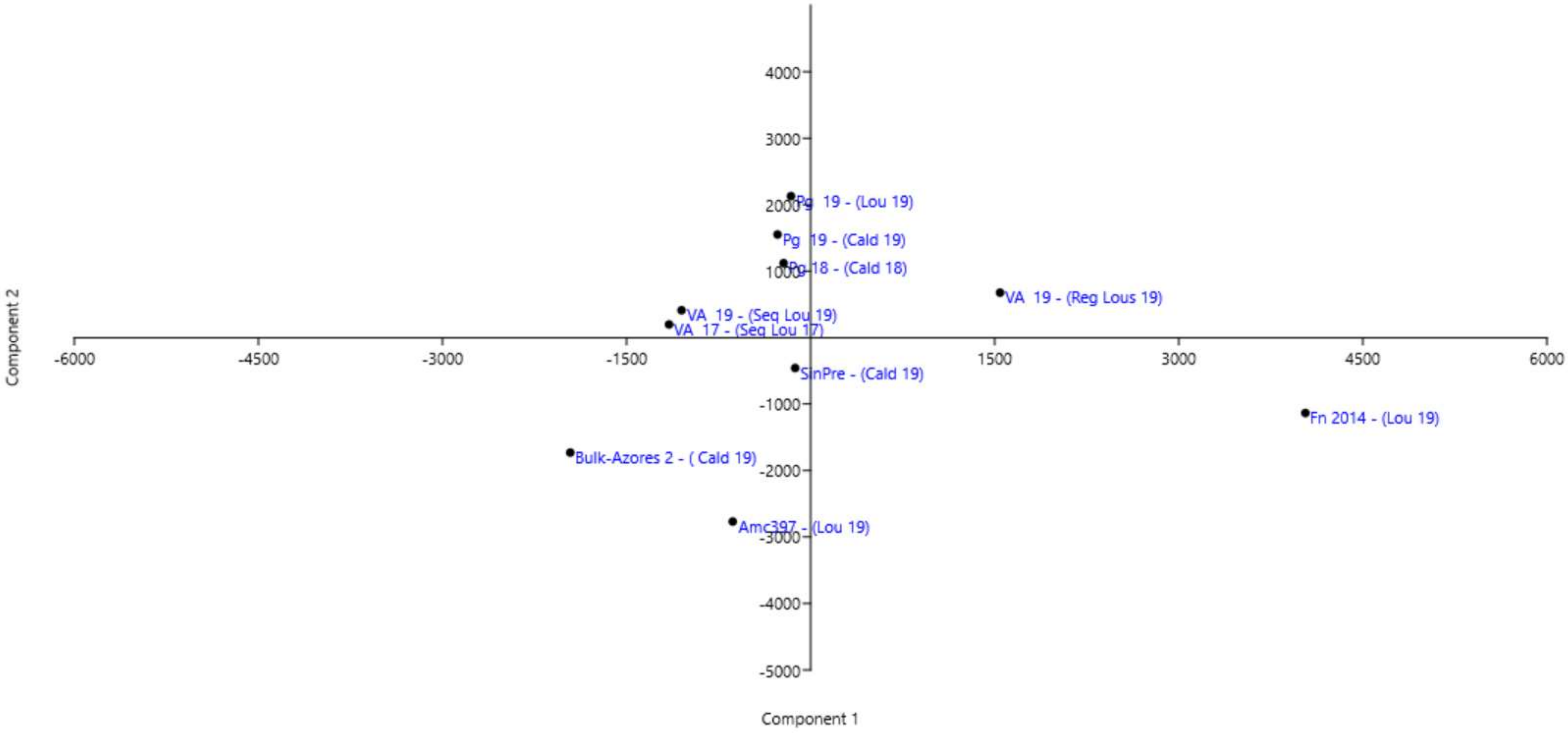


Figure 28- The PCA Analysis results for the genotypes average values of grain yield of the On-farm PPB trial in 2020 and 2021 (Components 1 and 2 explain 49,9 % and 41,24 %. of the variance detected, respectably).

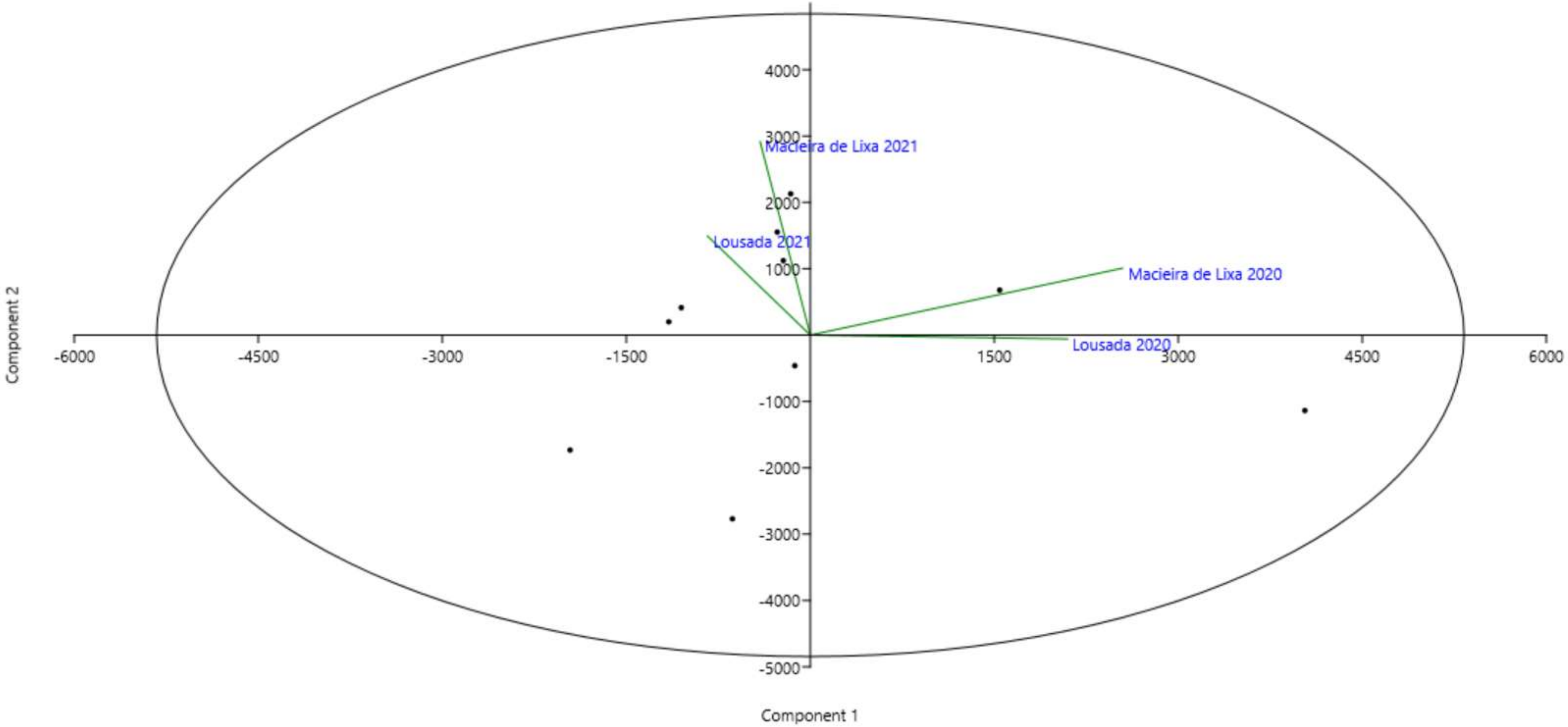


Figure 29- The results of the Biplot Analysis concerning genotypes and environments average scores of Grain yield of the On-farm PPB trial in 2020 and 2021 Components 1 and 2 explain 49,9 % and 41,24 %. of the variance detected, respectably).

Figure 30 displays a PCA and biplot analysis of grain yield values from both years of the trial. We can identify the entries Pg 19 - (Cald 19), VA 17 -(Seq Lou 17), Pg 19 -(Lou 19), and VA 19 -(Reg Lou 19) as having positive adaptation (score) to organic systems based on their greatest outcomes in relation to the tester Organic Caldeirão.

Comparisons of 'Pigarro' and 'Verdeal' maize populations from different years of mass selection in different environments (Figure 30) show no tendency in the selection environment more suitable for organic farming because the four maize populations identified as more adapted to the organic environment represent different selection environments. The majority of the detected populations, however, were selected in 2019, showing that the development of breeding efforts has been good in terms of adapting these gastronomic maize populations to low-input organic ecosystems.

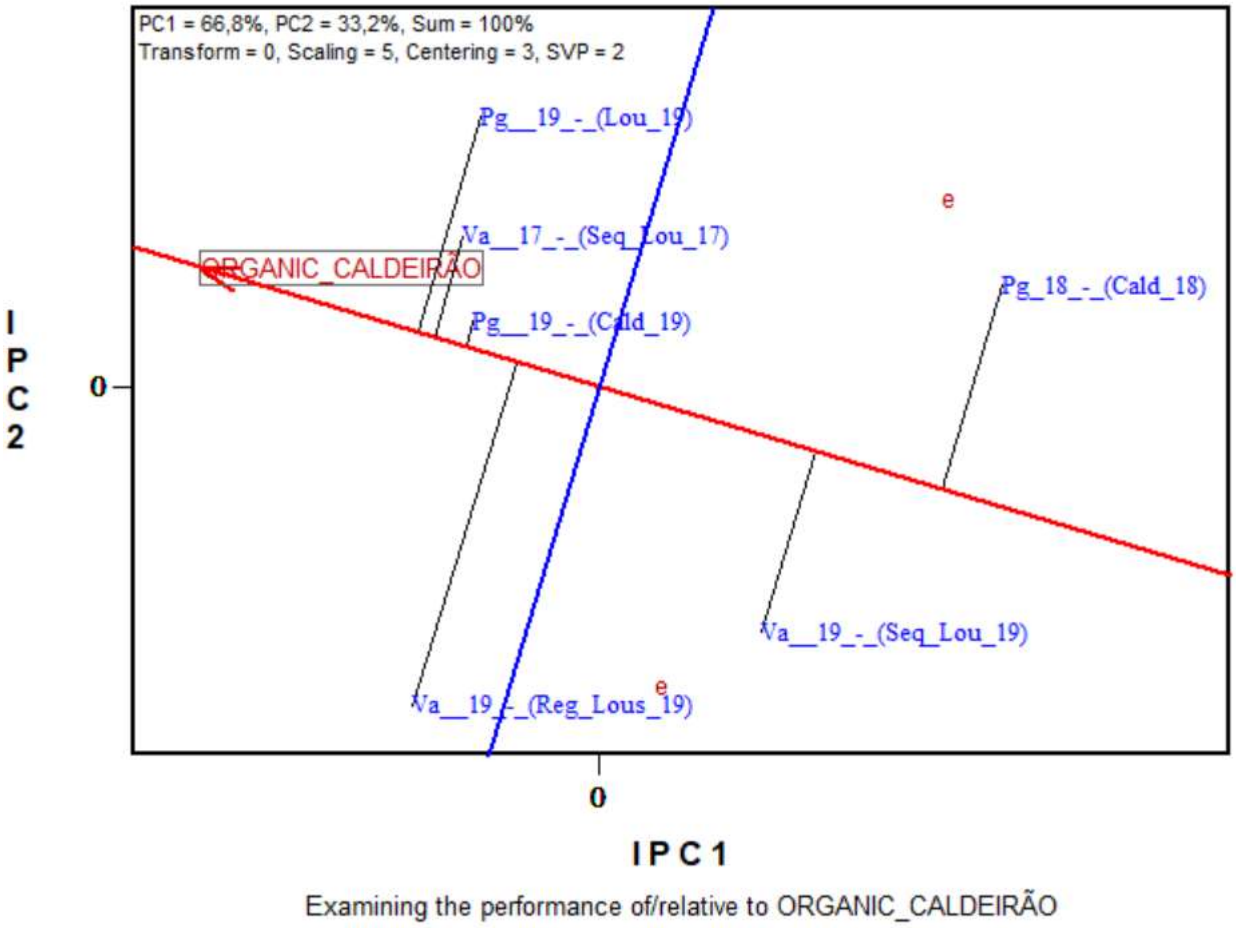


Figure 30- Biplot analysis of 6 Maize populations' performance (Grain yield) relative to the environment Organic Caldeirão (Components 1 and 2 explain 66,8 % and 33,2 %. of the variance detected, respectably).

3.8.3- Correlation between phenological parameters and Grain yield

The data from the On-Farm PPB trial (grain yield and HUNTERS descriptor) was correlated using Pearson's correlation (Table 29), and it was possible to identify factors with different significant positive correlations with grain yield in (H) Plant Hight, (H1E) first ear hight, (U) uniformity and ear placement (E).

Positive grain yield correlations imply that taller plants with better ear placement and uniformity produced more grain. The negative correlation between grain yield and leaf angle (N) can be explained by the low stand (plant density) on plots that were therefore less productive.

Overall, the results in some measure agreed with results found by other authors (Aziz et al., 1998; Bocanski et al., 2009; Duarte Pintado, 2019). They discovered significant, positive correlations between grain yield and plant height, as well as moderate correlations between first ear height and grain yield.

Table 34 Pearson's correlation with the data obtained from the characterization of plants according to the HUNTERS descriptor and Grain Yield. **Asterisk indicates statistical correlations** (*: P<0.05; **: P<0.01)

	Grain Yield	H	H1E	U	N	T	E	%R
H	,492**							
H1E	,373**	,894**						
U	,193*	0,147	,214*					
N	-0,033	-0,138	-0,053	0,027				
T	0,042	,252**	,226*	0,082	0,049			
E	,238**	,383**	,465**	0,023	,217*	-0,054		
%R	0,052	0,145	0,134	-,230*	0,143	0,094	0,092	
%S	-0,019	0,112	0,038	-0,144	0,104	-0,054	0,075	,363**

According to data collected in 2020 (Annexes IX and X) from biometric ear characterization and correlating with grain yield values via Pearson's correlation (Table 34), it was possible to identify the existence of significant and important correlations, which were signaled with a color gradient corresponding to the degree of correlation obtained between ear parameters and Grain Yield, such as:

- Length of the ear; L = 0.456.
- Ear weight; EW=0.513.
- Number of rows; NC= 0.471

Grain yield had the strongest genetic correlation with ear weight, followed by row count and ear length. The values obtained in our study agree with the findings of other authors (Bocanski et al., 2009; Duarte Pintado, 2019).

Table 35- Pearson's correlation with the data obtained from the characterization of ears according to the HUNTERS descriptor and Grain Yield (Yld15).

	Yld15	L	ED1	ED3	ED2	ED4	R1	R2	Fa	D/I	TR	CV	F/D	KC	EW	CW	KW15	KD	SW	HR	NC	DC1	DC3	DC2	DC4	M1	M2	Rq1	Rq2
L	0,456																												
ED1	0,151	-0,171																											
ED3	0,163	-0,115	,988**																										
ED2	0,136	-,228*	,957**	,926**																									
ED4	-0,007	-,233*	,431**	,407**	,446**																								
R1	0,251	-,293*	,763**	,701**	,780**	,344**																							
R2	0,261	-,285*	,759**	,697**	,774**	,348**	,998**																						
Fa	0,084	-,465**	,371**	,338**	,370**	0,079	,666**	,666**																					
D/I	0,039	-0,141	-,246*	-,254*	-,242*	-0,175	0,046	0,053	,365**																				
TR	0,007	0,06	,235*	,257*	0,186	0,135	,275*	,279*	0,14	-0,015																			
CV	-0,012	-,477**	,598**	,565**	,562**	,280*	,698**	,697**	,747**	0,09	,287*																		
F/D	-0,029	0,182	,218*	,264*	,225*	,220*	-,262*	-,258*	-,484**	-,349**	0,056	-0,206																	
KC	0,227	0,125	-0,087	-0,064	-0,11	-0,078	-,273*	-,264*	-0,16	-0,11	-0,206	-0,159	0,176																
EW	0,513	,558**	,613**	,652**	,591**	0,129	,345**	,346**	0,044	-0,156	0,169	0,089	,342**	0,043															
CW	0,007	-0,041	0,146	0,148	0,148	0,045	-0,075	-0,078	-0,076	-0,152	-0,053	0,034	,293*	-0,043	0,097														
KW15	0,444	,495**	,476**	,522**	,438**	0,057	0,139	0,139	-0,003	-0,125	0,113	0,05	,381**	0,025	,836**	,578**													
KD	0,121	0,183	0,171	0,118	,234*	0,177	0,139	0,127	-,304**	-,267*	-0,065	-0,155	0,127	0,014	,221*	-0,03	0,081												
SW	-0,014	0,159	0,098	0,195	0,006	-0,115	-,237*	-,229*	0,032	0,004	-0,105	0,092	,238*	,357**	,289*	-0,017	,288*	-,383**											
HR	0,46	,292*	,357**	,351**	,449**	0,155	,351**	,357**	-0,012	-,254*	0,087	0,036	0,188	-0,171	,606**	0,106	,441**	,262*	-0,007										
NC	0,471	,899**	-0,184	-0,129	-0,197	-0,203	-,289*	-,282*	-,382**	-0,068	0,113	-,452**	,254*	0,146	,572**	-0,066	,486**	0,106	0,199	,310**									
DC1	0,18	-,308**	,820**	,783**	,804**	,400**	,877**	,870**	,648**	-0,054	0,213	,734**	-0,206	-0,143	,360**	0,01	0,212	0,157	-0,066	,241*	-,338**								
DC3	0,171	-,318**	,755**	,737**	,720**	,261*	,810**	,803**	,668**	0,031	0,169	,701**	-,295*	-0,107	,318**	0,01	0,205	0,06	0,024	0,195	-,376**	,961**							
DC2	0,219	-,333**	,751**	,698**	,782**	,278*	,887**	,880**	,724**	0,029	0,105	,719**	-,265*	-0,136	,344**	0,029	0,208	0,156	-0,109	,268*	-,326**	,956**	,926**						
DC4	0,297	-,266*	,671**	,639**	,692**	0,186	,817**	,817**	,733**	0,148	0,062	,681**	-,299*	-0,092	,359**	0,037	,251*	0,067	0,021	,271*	-,267*	,893**	,919**	,949**					
M1	0,126	0,119	-,271*	-,296*	-,247*	-0,101	-0,08	-0,088	-0,039	,232*	-,461**	-0,171	-,313**	0,133	-0,108	-0,105	-0,117	,243*	-0,187	-,216*	0,09	-0,083	-0,074	-0,019	-0,019				
M2	0,134	0,161	-,326**	-,343**	-,313**	-0,149	-0,139	-0,146	-0,066	,284*	-,463**	-,217*	-,315**	0,153	-0,118	-0,103	-0,108	0,181	-0,139	-,244*	0,129	-0,148	-0,122	-0,083	-0,063	,989**			
Rq1	0,185	0,117	-,262*	-,282*	-,236*	-0,102	-0,117	-0,126	-0,119	0,147	-,512**	-0,188	-0,215	0,099	-0,131	-0,085	-0,144	,294*	-0,151	-0,161	0,116	-0,104	-0,099	-0,055	-0,057	,898**	,879**		
Rq2	0,16	0,193	-,246*	-,254*	-,246*	-0,104	-0,17	-0,154	-0,177	0,075	-,381**	-,227*	-0,186	,225*	-0,119	-0,093	-0,119	0,171	-0,076	-0,058	0,161	-0,138	-0,13	-0,137	-0,135	,672**	,664**	,758**	
CC	0,353	-,459**	-0,192	-0,199	-0,214	-0,048	-0,117	-0,125	,243*	,262*	-0,158	0,064	-,399**	0,122	-,534**	-,460**	-,602**	-,244*	0,007	-,594**	-,489**	0,038	0,125	0,021	0,011	0,125	0,126	0,086	0,104

Color gradient corresponding to the degree of correlation obtained between parameters											
-0,1	0,0	0,1	0,2	0,3	0,4	0,5	0,6	0,7	0,8	0,9	1,0

3.8.4- Multi-actor maize evaluations

The on-field evaluations conducted by farmers and stakeholders on October 15th were intended to reveal the farmers' and market (Stakeholders') preferences for the ten tested maize populations (Figure 31). However, due to COVID, attendance was lower than expected (4 participants), rendering the statistical analysis null and void. However, at this stage of cultivar demonstration and testing, feedback from farmers and stakeholders is critical. As an outcome, the three best scores in each morphological characteristic evaluated, as well as the best maize population suitable for maize bread production and animal feed, will be presented.

Figure 31- On-field evaluations in Macieira de Lixa trial site

The on-field evaluation scores ranged from 9 as I liked it very much to 1 as I disliked it a lot in only two characteristics: Plant Height and Grain type. Pg 19 - (Lou 19), SinPre - (Cald 19), and VA 17 - (Seq Lou 17) were chosen as the ideal plant height by the participants. Neither the significantly taller maize population (Fn 2014 - Lou 19) nor the significantly smaller maize population (Amc397 - Lou 19) previously identified in this work performed better in the on-field evaluation, indicating that participants prefer medium/tall plants.

Fn 2014 - (Lou 19), SinPre - (Cald 19), and Pg 18 - (Lou 19) had higher Uniformity scores (Cald 18).

Pg 19 - (Lou 19), SinPre - (Cald 19), and Pg 18 - (Cald 18) with the ideal ear height were chosen by the participants. Since the population SinPre - (Cald 19) (2nd best score) had significantly lower first ear height values (H1E) in the trial, the maize population's ear placement scores reveal a preference for lower first ear height values (H1E) (Figure 24).

The ear size scores on Fn 2014 - (Lou 19), Bulk-Azores 2 - (Cald 19), and Pg 18 - (Lou 19) revealed the preference. Fn 2014 – (Lou 19) is composed of the intercrossing of American elite inbred lines (dent and flint; 20% Portuguese and 80% American germplasm), and his trademark is the large kernel-row number and large ear size.

Participants selected Pg 19 - (Lou 19), Pg 18 - (Cald 18), and VA 19 - (Reg Lou 19) as the ideal grain type. Unveiling a trend toward white flint maize with maize bread ability, as all of the best-rated maize populations have those traits.When asked to choose the best maize population suitable for maize bread (Broa) production, all the participants identified Pg 19 - (Lou 19), mainly due to their higher number of rows, type, and color of the grain (Figure 32).

Figure 32- Ten ears of Pg 19 - (Lou 19) from Macieira de Lixa trial site

When asked to identify the best maize population suitable for animal feed, all participants chose Fn 2014 - (Lou 19), owing to higher biomass production (significantly taller maize population) and the length of the ears (Figure 33).

Figure 33- Five ears of Fn 2014 – (Lou 19) from Macieira de Lixa trial site

Finally, during the two years of the trial, the participatory breeding efforts (germplasm evaluation, on-field evaluation, technical support, meetings with farmers and stakeholders,...) expanded and strengthened the network of Sousa Valley farmers and municipal associations that, over the last ten years, have been developing the regional product and concept of "Broa de Milho do Vale do Sousa" (https://www.ptpt.pt/produtos/3) that only uses local maize populations (Landraces) in the production of maize bread (Ader-Sousa, 2018).

Chapter 4- General conclusions

The two trials cover the framework of Portuguese agriculture, in three realities or three agro-systems: Conventional Agriculture on large scale (Conventional Vagem), Organic Agriculture in a low-input environment (Organic Caldeirão), and Low-input Conventional agriculture (agro-ecological agriculture), very common in the northern part of Portugal where land is scarce and the large-scale inputs (synthetic fertilizers) and methodologies from conventional agriculture aren't suitable. This type of agriculture is often familiar, of self-sufficiency, and typically relies on animal manure as fertilizer.

The values from days-to-anthesis and days-to-silk in the first trial (Organic vs Conventional) reveal an unstable behavior of the maize populations between environments. Bringing the trial's diversity to life. The positive correlations between, on the one hand, days-to-anthesis and days-to-silk and, on the other hand, values of Root Lodging and Stalk lodging (Table 21) suggest that late sowing (June) in both years harmed maize populations with a longer crop cycle, exposing them to adverse weather conditions before harvest (October) and resulting in grain yield losses.

The maize populations tested were tall plants with a high biomass production.

The uniformity (U) values (HUNTERS scale) revealed yet again the high genetic diversity within the populations. Data on ear placement (E) revealed that they were inserted in the middle-upper part of the maize plants on average, implying a more difficult manual harvest and presenting future breeding goals when adapting to organic systems with a low level of mechanization. Despite this, they are adaptable to poly-cropping systems due to their high leaf angle (N) values, large tassels, and high ear placement, which makes them potentially suited and appealing to organic systems where poly-cropping is commonly used.

Because of the high level of fertilizer used in Conventional Vagem compared to Organic Caldeirão, the grain yield evaluation showed uneven behavior between environments, as expected. However, the Azorean maize populations 2516 – (Cald 19), 2501 – (Cald 19), and 2507 – (Cald 19) can be identified as the top three genotypes more suitable for low-input organic environments.

Apart from Amc397- (Lou 19), all maize populations tested in the second trial (On-Farm PPB) proved to be tall plants with high biomass production.

The HUNTERS scale values revealed the diversity within the populations studied (U). They also show adaptation to poly-cropping systems because of their high leaf angle (N) values, large tassels, and high ear placement.

The agronomic potential (Grain Yield) of the maize landraces tested is higher at the Macieira de Lixa site. With positive highlights to the average production of Fn-2014-(Cald 19), close to 10 Mg/ha (9 222 Kg/ha in 2020), the expected Grain Yield of most hybrids (High levels of synthetic fertilization) in Portugal ranges from 12 Mg/ha to 16 Mg/ha (Agrotec, 2018).

The two maize Landraces with gastronomic aptitude ('Pigarro' and 'Verdeal') had the best overall (2-year) performance according to Grain Yield Rank (Table 33). The selection environment was critical in determining the best rank performance in each environment (Macieira and Lousada). However, no trend was seen in the selection of the optimal selection environment when the Grain Yield data of the 6 maize populations with maize bread aptitude was studied (Figure 31).

The germplasm study and discussions with farmers and stakeholders allowed for the sharing of knowledge and analysis required to identify new breeding targets while simultaneously continuing PPB operations in Sousa Valley. This multi-actor strategy was critical for knowledge sharing and retaining farmers and stakeholders motivated and interested.

Finally, the on-field evaluation will be repeated in next year's maize campaign, with more participants (ideally) to incorporate a statistical view of farmers and market options.

References

AccuWeather. (2020). *Meteorologia mensal em Macieira da Lixa, Porto, Portugal.* https://www.accuweather.com/pt/pt/macieira-da-lixa/863135/november-weather/863135?year=2020

Ader-Sousa. (2018). *Caracterização do território - Ader-Sousa.* https://www.adersousa.pt/dlbc-rural/eld/caracterizacao-do-territorio/

Agrotec. (2018). *Área de milho aumenta em Portugal.* http://www.agrotec.pt/noticias/area-de-milho-aumenta-em-portugal/

Alves, M. L., Belo, M., Carbas, B., Brites, C., Paulo, M., Mendes-Moreira, P., Brites, C., Bronze, M. do R., Šatović, Z., & Vaz Patto, M. C. (2018). Long-term on-farm participatory maize breeding by stratified mass selection retains molecular diversity while improving agronomic performance. *Evolutionary Applications*, *11*(2), 254–270. https://doi.org/10.1111/eva.12549

Alves, M. L., Carbas, B., Gaspar, D., Paulo, M., Brites, C., Mendes-Moreira, P., Brites, C. M., Malosetti, M., Van Eeuwijk, F., & Vaz Patto, M. C. (2019). Genome-wide association study for kernel composition and flour pasting behavior in wholemeal maize flour. *BMC Plant Biology*, *19*(1), 1–17. https://doi.org/10.1186/s12870-019-1729-7

Andjelkovic, V., Babic, V., & Kravic, N. (2017). Genetic resources in maize breeding. *Selekcija i Semenarstvo*, *23*(1), 37–48. https://doi.org/10.5937/selsem1701037a

Andjelkovic, V., & Polje, Z. (2016). *Maize Genetic Resource . indd. June.*

Andorf, C., Beavis, W. D., Hufford, M., Smith, S., Suza, W. P., Wang, K., Woodhouse, M., Yu, J., & Lübberstedt, T. (2019). Technological advances in maize breeding: past, present and future. In *Theoretical and Applied Genetics* (Vol. 132, Issue 3). Springer Berlin Heidelberg. https://doi.org/10.1007/s00122-019-03306-3

Anpromis. (2020). Evolução das áreas declaradas por cultura - Total Nacional (2004 a 2020). *Anpromis*, 2020. http://www.anpromis.pt/images/dados/Evol_CA_2004_2020_Total-Nacional.pdf

Aziz, K., Rehman, A., & Rauf, A. (1998). Heritability and Interrelationships for Some Plant Traits in Maize Single Crosses. *Pakistan Journal of Biological Sciences*, *1*(4), 313–314. https://doi.org/10.3923/pjbs.1998.313.314

Bioversity International. (2007). Developing crop descriptor lists. *Bioversity*, *13*, 1–84. www.bioversityinternational.org

Bioversity International. (2019). *The Alliance Annual Report 2019.* https://annualreport2019.alliancebioversityciat.org/

Bioversity International. (2020). *Conservation of local crops and their wild relatives.* https://www.bioversityinternational.org/research-portfolio/conservation-of-crop-diversity/

Bocanski, J., Sreckov, Z., & Nastasic, A. (2009). Genetic and phenotypic relationship between grain yield and components of grain yield of maize (Zea mays L.). *Genetika*, *41*(2), 145–154. https://doi.org/10.2298/gensr0902145b

Burle, M. L., & Oliveira, M. do S. P. de. (2010). Manual de Curadores de Germoplasma – Vegetal: Caracterização Morfológica. *Embrapa Documentos*, 16.

Cancellier, L. L., Afférri, F. S., Cruz, O. S., Peluzio, J. M., & Melo, A. V. de. (2011). Desempenho de populações de milho para produção de grãos e consumo in natura no Estado de Tocantins. *Revista Ciência Agronômica*, *42*(2), 542–553. https://doi.org/10.1590/s1806-66902011000200038

Carena, M. J. (2013). Development of cold and drought tolerant short-season maize germplasm for fuel and feed utilization. *Crop Breeding and Applied Biotechnology*, *13*(1), 1–8. https://doi.org/10.1590/s1984-70332013000100001

Ceccarelli, S. (2012). *Plant breeding with farmers - a technical manual*.

Ceccarelli, Salvatore, & Grando, S. (2019). Participatory plant breeding: Who did it, who does it and where? *Experimental Agriculture*. https://doi.org/10.1017/S0014479719000127

Climate. (2021). *Temperatura, Tempo e Dados climatológicos - Climate-Data.org*. 2021. https://pt.climate-data.org/europa/portugal/coimbra/coimbra-160/

Colley, M. R., Dawson, J. C., McCluskey, C., Myers, J. R., Tracy, W. F., & Lammerts van Bueren, E. T. (2021). Exploring the emergence of participatory plant breeding in countries of the Global North – a review. *The Journal of Agricultural Science*, 1–19. https://doi.org/10.1017/s0021859621000782

Costa-Rodrigues, L. (1971). Races of maize in Portugal. *Agronomia Lusitana*, *31*(4), 239–284. https://eurekamag.com/research/000/174/000174812.php

Crespo-Herrera, L. A., & Ortiz, R. (2015). Plant breeding for organic agriculture: Something new? *Agriculture and Food Security*, *4*(1), 1–7. https://doi.org/10.1186/S40066-015-0045-1/METRICS

Crow, J. F. (1998). 90 Years Ago: the Beginning of Hybrid Maize. *Genetics*, *148*(3), 923–928. https://doi.org/10.1093/genetics/148.3.923

Cuena-Lombraña, A., Sanna, M., Porceddu, M., & Bacchetta, G. (2020). Does storage under gene bank conditions affect seed germination and seedling growth? The case of Senecio morisii (asteraceae), a vascular plant exclusive to sardinian water meadows. In *Plants* (Vol. 9, Issue 5). https://doi.org/10.3390/plants9050581

de Araújo, P. M., & Nass, L. L. (2002). Characterization and evaluation of maize landraces. *Characterization and Evaluation of Maize Landraces*, *59*(3), 589–593. https://doi.org/10.1590/S0103-90162002000300027

Direção Geral de Agricultura e Pescas de Lisboa e Vale do Tejo. (2012). *Quantidade e composição média de Estrumes e Chorumes não diluídos, produzidos anualmente por diferentes espécies pecuárias e sua conversão em cabeça normal (CN)* (Vol. 52, Issue 8). http://www.drapn.min-agricultura.pt/drapn/conteudos/zv/codigobpa.pdf

DIVERSIFOOD. (2016). *Mission – Diversifood*. http://www.diversifood.eu/mission/

Doebley, J. F., Gaut, B. S., & Smith, B. D. (2006). The Molecular Genetics of Crop Domestication. In *Cell* (Vol. 127, Issue 7, pp. 1309–1321). https://doi.org/10.1016/j.cell.2006.12.006

Duarte Pintado. (2019). *Mestrado em Engenharia Agropecuária Relatório de Estágio Profissionalizante Mestrado em Engenharia Agropecuária Relatório de Estágio Profissionalizante*.

Ebert, A. W., & Engels, J. M. M. (2020). Plant biodiversity and genetic resources matter! In *Plants* (Vol. 9, Issue 12). https://doi.org/10.3390/plants9121706

ECPGR. (2017). *ECPGR Concept for on-farm conservation and management of plant genetic resources for food and agriculture, Rome, Italy*.

ESAC. (2020). *Estação Meteorológica da Escola Superior Agrária de Coimbra – Valores Médios Mensais De Temperatura E Pluviometria. Coimbra: 2020*. https://doi.org/10.14210/alcance.v24n1.p001

European Commission. (2021). *Organic Action Plan | European Commission*. European Commission. https://ec.europa.eu/info/food-farming-fisheries/farming/organic-farming/organic-action-plan_en

FAO. (2003). Weighing the GMO arguments: against. In *FAO News room*. https://www.fao.org/english/newsroom/focus/2003/gmo8.htm

Ferrão, J. E. M. (1992). *A Aventura das Plantas e os Descobrimentos Portugueses. Programa Nacional de Edições Comemorativas dos Descobrimentos Portugueses, 1992. - Pesquisa Google*. https://www.google.com/search?q=FERRÃO%2C+J.E.M.+-+A+Aventura+das+Plantas+e+os+Descobrimentos+Portugueses.+Programa+Nacional+de+Edições+Comemorativas+dos+Descobrimentos+Portugueses%2C+1992.&rlz=1C1GCEA_enPT955PT956&oq=FERRÃO%2C+J.E.M.+-+A+Aventura+das+Pla

Frederic Rey, P. R., (ITAB), E. F., (LBI), A. de B., (ÖMKi), J. F., (ORC), A. C., & (FiBL-CH), M. L. (2021). *Frugal , multi-actor and decentralised cultivar evaluation models for organic agriculture: methods , tools and guidelines*.

Fuss, A., Kovacs, T., Pedersen, T. M., Raaijmakers, M., Schafer, F., Gatzert, X., Bruhl, K., Bocci, R., & Petitti, M. (2018). *How to implement the organic regulation to increase production & use of organic seed*. 1–24.

Gotor, E., Alercia, A., Rao, V. R., Watts, J., & Caracciolo, F. (2008). The scientific information activity of Bioversity International: The descriptor lists. *Genetic Resources and Crop Evolution*, *55*(5), 757–772. https://doi.org/10.1007/s10722-008-9342-x

Guna, A., Zhang, J., Tong, S., Bao, Y., Han, A., & Li, K. (2019). Effect of climate change on maize yield in the growing season: A case study of the Songliao Plain maize belt. *Water (Switzerland)*, *11*(10). https://doi.org/10.3390/w11102108

Hallauer; A., R. (1994). Corn Genetcis and Breeding. *Dia of Agricultural Science*, 455–467.

Hallauer, A. R. (2007). History, Contribution, and Future of Quantitative Genetics in Plant Breeding: Lessons From Maize. *Crop Science*, *47*(SUPPL. DEC.), S-4. https://doi.org/10.2135/CROPSCI2007.04.0002IPBS

Hossain, F., Bhat, S. R., Mohapatra, T., & Singh, A. K. (2019). Genetics on a maize cob: A teaching tool for schools. *Indian Journal of Genetics and Plant Breeding (The)*, *79*(01S), 340–366. https://doi.org/10.31742/ijgpb.79s.1.27

Husaini, A. M., & Sohail, M. (2018). Time to redefine organic agriculture: Can't GM crops be certified as organics? *Frontiers in Plant Science*, *9*, 423. https://doi.org/10.3389/FPLS.2018.00423/BIBTEX

ICAR. (2021). *India Maze Scenario – ICAR-Indian Institute of Maize Research*. https://iimr.icar.gov.in/world-maze-scenario/

IFOAM. (2021). *The Four Principles of Organic Agriculture | IFOAM*. IFOAM - Organics International. https://www.ifoam.bio/why-organic/shaping-agriculture/four-principles-organic

INE. (2020). *Estatísticas Agrícolas 2020*.

IPGRI. (2000). *IPGRI*. https://www.bioversityinternational.org/fileadmin/bioversity/publications/Web_version/572

/ch01.htm

IPMA, I. P. do M. e da A. (2021). *IPMA - Observação de superfície horária com gráficos*. https://www.ipma.pt/pt/otempo/obs.superficie/

Josia, C., Mashingaidze, K., Amelework, A. B., Kondwakwenda, A., Musvosvi, C., & Sibiya, J. (2021). SNP-based assessment of genetic purity and diversity in maize hybrid breeding. *PLoS ONE*, *16*(8 August), 1–15. https://doi.org/10.1371/journal.pone.0249505

Karaman, M., Caglayan, K., & Gazel, M. (2021). *NEW APPROACHES IN CEREAL* (Issue September).

Kottek, M., Grieser, J., Beck, C., Rudolf, B., & Rubel, F. (2006). World map of the Köppen-Geiger climate classification updated. *Meteorologische Zeitschrift*, *15*(3), 259–263. https://doi.org/10.1127/0941-2948/2006/0130

Kovács, T., & Pedersen, T. M. (2019). *Deliverable 2.1: Overview on the current organizational models for cultivar testing for Organic Agriculture over some EU countries*. *May*, 84. https://www.liveseed.eu/wp-content/uploads/2019/07/LIVESEED_D2.1_Overview-of-the-organisational-models-of-cultivar-trials-for-organic-agriculture.pdf

Kutka, F. (2011). Open-pollinated vs. hybrid maize cultivars. *Sustainability*, *3*(9), 1531–1554. https://doi.org/10.3390/su3091531

Leff, B., Ramankutty, N., & Foley, J. A. (2004). Geographic distribution of major crops across the world. *Global Biogeochemical Cycles*, *18*(1). https://doi.org/10.1029/2003GB002108

LIVESEED. (2020). *Objectives and aims of LIVESEED: boosting organic seed/plant breeding*. https://www.liveseed.eu/about-liveseed/objectives-aims-liveseed/

Love, L. B. (2021). *Modeling the Early History of Maize in the North American Southwest*. *April*.

Matsuoka, Y., Vigouroux, Y., Goodman, M. M., Sanchez, J. G., Buckler, E., & Doebley, J. (2002). A single domestication for maize shown by multilocus microsatellite genotyping. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(9), 6080–6084. https://doi.org/10.1073/pnas.052125199

McLean-Rodríguez, F. D., Camacho-Villa, T. C., Almekinders, C. J. M., Pè, M. E., Dell'Acqua, M., & Costich, D. E. (2019). The abandonment of maize landraces over the last 50 years in Morelos, Mexico: a tracing study using a multi-level perspective. *Agriculture and Human Values*, *36*(4), 651–668. https://doi.org/10.1007/S10460-019-09932-3

Mendes-Moreira. (2015). *Effectiveness of Selection Methods for Improvement of Portuguese Maize (Zea mays L .) The study of fasciation* (Issue July).

Mendes-Moreira, Dinis, I., & Santos, D. (2018). *Valorização de recursos genéticos endógenos através das sinergias multi-ator: O caso do programa VASO 2.0*. *December*.

Mendes-Moreira, Mendes-Moreira, J., Fernandes, A., Andrade, E., Hallauer, A. R., Pêgo, S. E., & Vaz Patto, M. C. (2014). Is ear value an effective indicator for maize yield evaluation? *Field Crops Research*, *161*, 75–86. https://doi.org/10.1016/j.fcr.2014.02.015

Mendes-Moreira, Satovic, Z., Mendes-Moreira, J., Santos, J. P., Nina Santos, J. P., Pêgo, S., & Vaz Patto, M. C. (2017). Maize participatory breeding in Portugal: Comparison of farmer's and breeder's on-farm selection. *Plant Breeding*, *136*(6), 861–871. https://doi.org/10.1111/PBR.12551

Mendes-Moreira, Vaz Patto, M. C., Mota, M., Mendes-Moreira, J., Santos, J. P. N., Santos, J. P. P., Andrade, E., Hallauer, A. R., & Pego, S. E. (2009). "Fandango": Long term adaptation of exotic germplasm to a Portuguese on-farm-conservation and breeding project. *Maydica*, *54*(2–3), 269–285.

Menz, J., Modrzejewski, D., Hartung, F., Wilhelm, R., & Sprink, T. (2020). Genome Edited Crops Touch the Market: A View on the Global Development and Regulatory Environment. *Frontiers in Plant Science*, *11*(October), 1–17. https://doi.org/10.3389/fpls.2020.586027

Moreira, P. M. R. M. (2006). Participatory maize breeding in Portugal. A case study. *Acta Agronomica Hungarica*, *54*(4), 431–439. https://doi.org/10.1556/AAgr.54.2006.4.6

Nass, L. L., & Paterniani, E. (2000). Pre-breeding: a link between genetic resources and maize breeding. *Scientia Agricola*, *57*(3), 581–587. https://doi.org/10.1590/s0103-90162000000300035

Njagi, T. (2019). Understanding the political economy of maize in Kenya. In *The conversation; Academic rigour, journalistic flair.* (p. 53). https://theconversation.com/understanding-the-political-economy-of-maize-in-kenya-120336

Ortiz, R., Taba, S., Chávez Tovar, V. H., Mezzalama, M., Xu, Y., Yan, J., & Crouch, J. H. (2010). Conserving and enhancing maize genetic resources as global public goods-A perspective from CIMMYT. *Crop Science*, *50*(1), 13–28. https://doi.org/10.2135/cropsci2009.06.0297

Pêgo. (1982). *Genetic potential of Portuguese maize germplasm with abnormal ear shape*.

Pêgo, S., & Hallauer, A. R. (1984). Portuguese maize germplasm with abnormal ear shape. *Maydica*, *29*, 39–53.

Perales H. R., Brush S. B., C. O. Q. (2003). *LANDRACES OF MAIZE IN CENTRAL MEXICO: AN ALTITUDINAL TRANSECT*. *57*(1), 7–20.

Peres, S. (2016). Saving the gene pool for the future: Seed banks as archives. *Studies in History and Philosophy of Science Part C:Studies in History and Philosophy of Biological and Biomedical Sciences*, *55*, 96–104. https://doi.org/10.1016/j.shpsc.2015.09.002

Plecher, H. (2021). • *Average prices for sugar worldwide from 2014 to 2025 | Statista*. Statista. https://www.statista.com/statistics/675820/average-prices-maize-worldwide/

Revilla, P., Alves, M. L., Andelković, V., Balconi, C., Dinis, I., Mendes-Moreira, P., Redaelli, R., Ruiz de Galarreta, J. I., Vaz Patto, M. C., Žilić, S., & Malvar, R. A. (2022). Traditional Foods From Maize (Zea mays L.) in Europe. *Frontiers in Nutrition*, *8*(January). https://doi.org/10.3389/fnut.2021.683399

Serpolay, E., Nuijten, E., & Rossi, A. (2018). *Building blocks to develop multi-actor research projects and examples of community based plant breeding and management of agrobiodiversity from Diversifood experience*.

Serrano, P., & Ges, A. (2016). *O Sector da Produção de Milho Enquadramento*.

Serratos Hernández, J. A. (2009). *The origin and diversity of maize in the american continent*. 37. http://www.cropwildrelatives.org/fileadmin/www.cropwildrelatives.org/documents/Origin and diversity of maize.pdf

Shahbanden, M. (2019). • *Corn production by country 2018/19 | Statista*. Ststista. https://www.statista.com/statistics/254292/global-corn-production-by-country/

Sharma, S., & Carena, M. J. (2016). Grain quality in Maize (Zea mays L.): breeding implications for short-season drought environments. *Euphytica*, *212*(2), 247–260. https://doi.org/10.1007/s10681-016-1764-5

Sharma, Shivali. (2017). Prebreeding using wild species for genetic enhancement of grain legumes at ICRISAT. *Crop Science*, *57*(3), 1132–1144. https://doi.org/10.2135/cropsci2017.01.0033

Silveira, D. L., Filho, A. C., Neu, I. M. M., De Souza, J. M., Kleinpaul, J. A., & Dumke, G. E. (2021). Genetic divergence in maize regarding grain yield and tassel traits1. *Revista Ciencia Agronomica*, *52*(4), 1–11. https://doi.org/10.5935/1806-6690.20210060

Solfanelli, F., Ozturk, E., Zanoli, R., Orsini, S., & Schäfer, F. (2019). *The State of Organic Seed in Europe* (pp. 1–28). www.liveseed.eu

SOLIBAN. (2015). *www . solibam . eu*. 7–8.

Stevens, T., & Madani, K. (2016). Future climate impacts on maize farming and food security in Malawi. *Scientific Reports*, *6*(October), 1–14. https://doi.org/10.1038/srep36241

The British Museum. (2020). *Teaching History with 100 Objects*. http://www.teachinghistory100.org/objects/about_the_object/maize_god

Vaz Patto, M. C., Moreira, P. M., Almeida, N., Satovic, Z., & Pego, S. (2008). Genetic diversity evolution through participatory maize breeding in Portugal. *Euphytica*, *161*(1–2), 283–291. https://doi.org/10.1007/s10681-007-9481-8

Vernooy, R., Mulesa, T. H., Gupta, A., Jony, J. A., Koffi, K. E., Mbozi, H., Singh, P. B., Shrestha, P., Tjikana, T. T., & Wakkumbure, C. L. K. (2020). The role of community seed banks in achieving farmers' rights. *Development in Practice*, *30*(5), 561–574. https://doi.org/10.1080/09614524.2020.1727415

WOLFF, F. DE. (1972). *Mass Selection in Maize Composites By Means of. 1.*

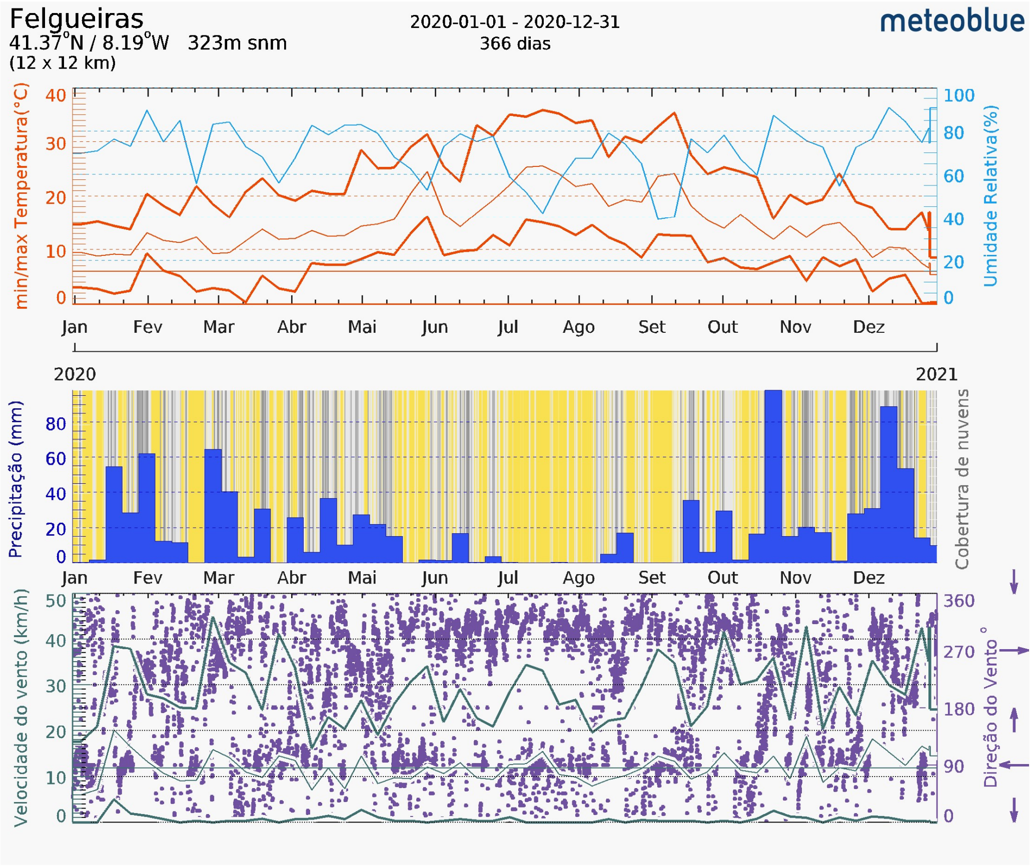
Xue, J., Ming, B., Xie, R., Wang, K., Hou, P., & Li, S. (2020). Evaluation of maize lodging resistance based on the critical wind speed of stalk breaking during the late growth stage. *Plant Methods*, *16*(1), 1–12. https://doi.org/10.1186/S13007-020-00689-Z/FIGURES/10

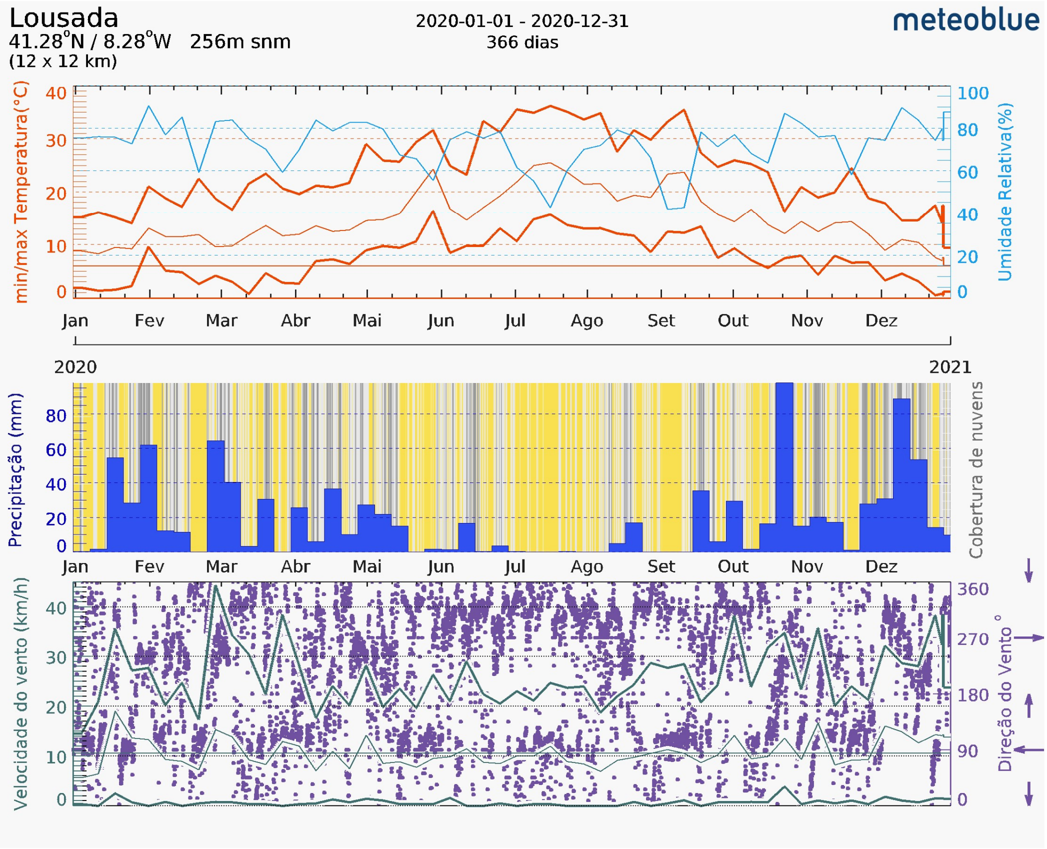
Annexes

Annex I –Weather Data Coimbra (Esac- Bencanta weather station)

Mês	Temperatura (ºC)								Humidade relativa (%)	Radiação Global (MJ m^{-2} d^{-1})	Vento		Precipitação total (mm)	Precipitação max. Diária (mm/dia)	Evapotranspiração de Referência (mm)
	med	mx. m	mn. m	mx. a	mn. a	Horas de frio (T<7,2 ºC)	Relva				Velocidade média (km/ h)	Direção			
							mn. m	mn. a							
jan/20	10,7	15,7	7,1	18,6	-0,4	161	-	-	84,3	8	5,1	S	49,8	11,2	29,5
fev/20	13,1	19,3	8,2	26	1,5	68	-	-	86,5	11,3	2,9	SE	33	10,6	40,7
mar/20	13,1	18,7	8,3	25,3	2,3	44	-	-	83,1	14,6	3,9	NW	86	43,2	64,5
abr/20	14,7	19,6	10,5	23,5	3,2	-	-	-	86,7	14,4	3,5	NW	90,2	15	71
mai/20	19,2	26,2	13,4	35,1	10,6	-	-	-	80,4	22,5	3	NW	56,4	17,4	124
jun/20	19,6	26	13,8	33,6	7,9	-	-	-	77,4	23,1	3	NW	7,4	4,6	126
jul/20	23,7	33,4	16,3	40,3	9,9	-	-	-	71	27,1	3	NW	6,2	6	167,3
ago/20	21,7	29,4	16,3	34,4	8,8	-	-	-	77,8	22,9	3,1	NW	24,6	21,4	133,2
set/20	20,9	29,8	14,1	39	9,3	-	-	-	74,6	18,2	2,7	NW	39,8	18	98,8
out/20	15,9	21,9	10,9	27,4	5	-	-	-	85,3	12,7	3	NW	143,6	44,4	56
nov/20	14,6	20,2	10,3	26,9	5,4	11	-	-	86,4	8,5	3,2	SE	76,2	28	32,2
dez/20	11	15,3	7,1	20,9	-0,3	146	-	-	91	6,9	2,7	W	153,2	21,8	17,8

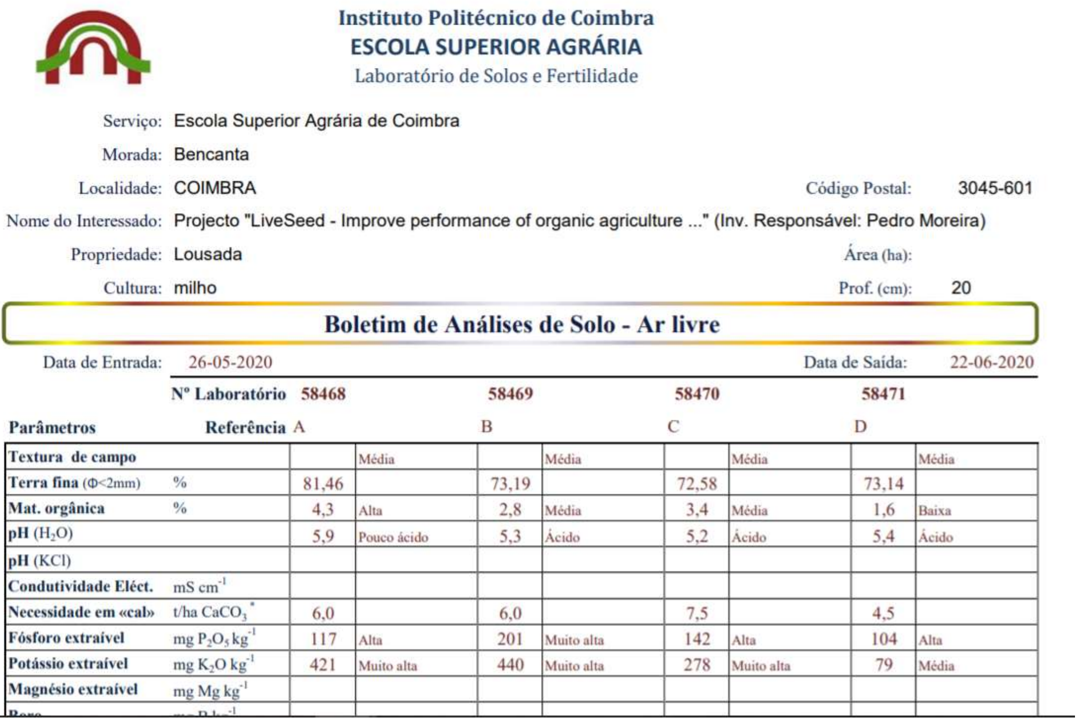
Annex II –Weather Data Felgueiras e lousada (Meteo blue)





Annex III Soil Chemical Analysis 2020

A- Lousada ; G-Macieira de Lixa; H- Conventional Vagem; I- Organic Caldeirão



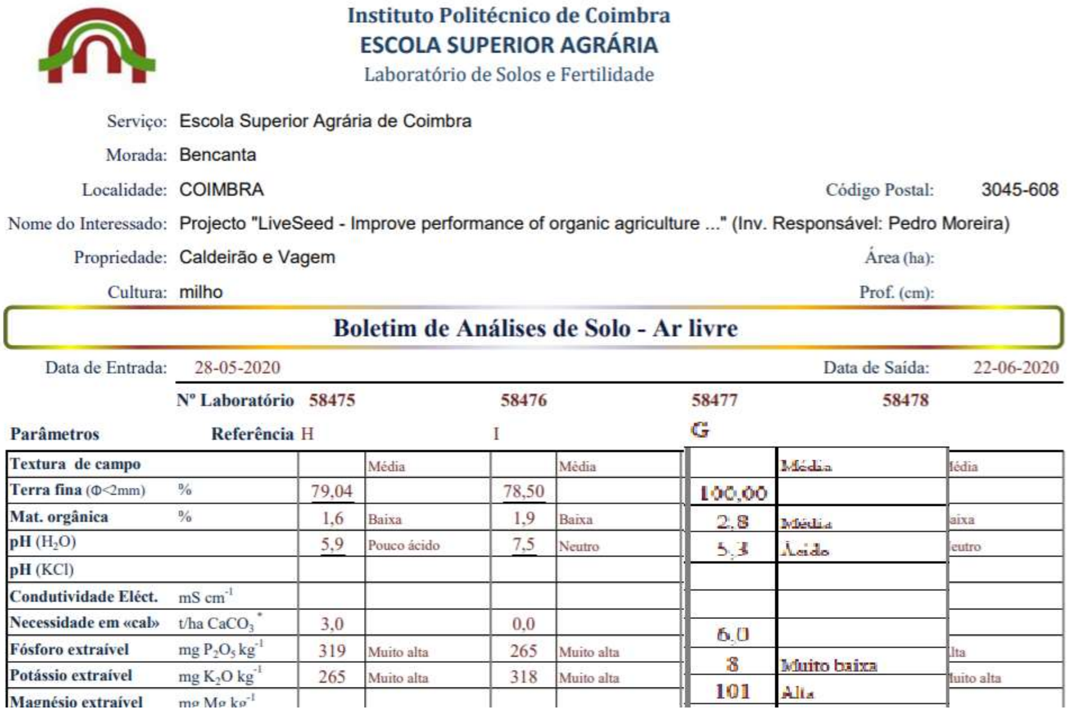
Instituto Politécnico de Coimbra
ESCOLA SUPERIOR AGRÁRIA
Laboratório de Solos e Fertilidade

Serviço: Escola Superior Agrária de Coimbra
Morada: Bencanta
Localidade: COIMBRA Código Postal: 3045-601
Nome do Interessado: Projecto "LiveSeed - Improve performance of organic agriculture ..." (Inv. Responsável: Pedro Moreira)
Propriedade: Lousada Área (ha):
Cultura: milho Prof. (cm): 20

Boletim de Análises de Solo - Ar livre

Data de Entrada: 26-05-2020 Data de Saída: 22-06-2020

	Nº Laboratório	**58468**		**58469**		**58470**		**58471**	
Parâmetros	**Referência**	A		B		C		D	
Textura de campo			Média		Média		Média		Média
Terra fina (Φ<2mm)	%	81,46		73,19		72,58		73,14	
Mat. orgânica	%	4,3	Alta	2,8	Média	3,4	Média	1,6	Baixa
pH (H_2O)		5,9	Pouco ácido	5,3	Ácido	5,2	Ácido	5,4	Ácido
pH (KCl)									
Condutividade Eléct.	mS cm^{-1}								
Necessidade em «cal»	t/ha $CaCO_3$ *	6,0		6,0		7,5		4,5	
Fósforo extraível	mg P_2O_5 kg^{-1}	117	Alta	201	Muito alta	142	Alta	104	Alta
Potássio extraível	mg K_2O kg^{-1}	421	Muito alta	440	Muito alta	278	Muito alta	79	Média
Magnésio extraível	mg Mg kg^{-1}								
Boro	mg B kg^{-1}								



Instituto Politécnico de Coimbra
ESCOLA SUPERIOR AGRÁRIA
Laboratório de Solos e Fertilidade

Serviço: Escola Superior Agrária de Coimbra
Morada: Bencanta
Localidade: COIMBRA Código Postal: 3045-608
Nome do Interessado: Projecto "LiveSeed - Improve performance of organic agriculture ..." (Inv. Responsável: Pedro Moreira)
Propriedade: Caldeirão e Vagem Área (ha):
Cultura: milho Prof. (cm):

Boletim de Análises de Solo - Ar livre

Data de Entrada: 28-05-2020 Data de Saída: 22-06-2020

	Nº Laboratório	**58475**		**58476**		**58477**		**58478**	
Parâmetros	**Referência**	H		I		G			
Textura de campo			Média		Média		Média		lédia
Terra fina (Φ<2mm)	%	79,04		78,50		100,00			
Mat. orgânica	%	1,6	Baixa	1,9	Baixa	2,8	Média		aixa
pH (H_2O)		5,9	Pouco ácido	7,5	Neutro	5,3	Ácido		eutro
pH (KCl)									
Condutividade Eléct.	mS cm^{-1}								
Necessidade em «cal»	t/ha $CaCO_3$ *	3,0		0,0		6,0			
Fósforo extraível	mg P_2O_5 kg^{-1}	319	Muito alta	265	Muito alta	8	Muito baixa		lta
Potássio extraível	mg K_2O kg^{-1}	265	Muito alta	318	Muito alta	101	Alta		luito alta
Magnésio extraível	mg Mg kg^{-1}								

Annex IV Soil Chemical Analysis 2021

A-Macieira de Lixa;B- Conventional Vagem; C- Organic Caldeirão; D- Lousada

esac
Politécnico de Coimbra

laboratório de
solos e fertilidade

Serviço: Escola Superior Agrária de Coimbra
Morada: Bencanta
Localidade: COIMBRA **Código Postal:** 3045-601
Nome do Interessado: PDR 2020 - Eng.º Pedro Moreira
Propriedade: **Área (ha):**
Cultura: **Prof. (cm):**

Boletim de Análises de Solo - Ar livre

Data de Entrada: 02-06-2021 **Data de Saída:** 18-06-2021

Nº Laboratório 59864 59865 59866

Parâmetros	**Referência**	Parcela A		Parcela B		Parcela C			
Textura de campo			Média		Média		Média		
Terra fina (Φ<2mm)	%	95,03		88,74		82,04			
Mat. orgânica	%	2,8	Média	2,2	Média	3,5	Média		
pH (H_2O)		5,7	Pouco ácido	6,2	Pouco ácido	6,0	Pouco ácido		
pH (KCl)									
Condutividade Eléct.	mS cm^{-1}								
Necessidade em «cal»	t/ha $CaCO_3$*	4,0		2,0		5,0			
Fósforo extraível	mg P_2O_5 kg^{-1}	19	Muito baixa	78	Média	96	Média		
Potássio extraível	mg K_2O kg^{-1}	194	Alta	370	Muito alta	280	Muito alta		
Magnésio extraível	mg Mg kg^{-1}								

esac
Politécnico de Coimbra

laboratório de
solos e fertilidade

Serviço: Escola Superior Agrária de Coimbra
Morada: Bencanta
Localidade: COIMBRA **Código Postal:** 3045-601
Nome do Interessado: Projecto "LiveSeed - Improve performance of organic agriculture ..." (Inv. Responsável: Pedro Moreira)
Propriedade: **Área (ha):**
Cultura: **Prof. (cm):**

Boletim de Análises de Solo - Ar livre

Data de Entrada: 11-06-2021 **Data de Saída:** 18-06-2021

Nº Laboratório 59878 59879 59880

Parâmetros	**Referência**	Parcela D		Parcela E		Parcela F			
Textura de campo			Média		Média		Média		
Terra fina (Φ<2mm)	%	79,12		78,50		76,71			
Mat. orgânica	%	1,9	Baixa	2,3	Média	2,0	Baixa		
pH (H_2O)		7,2	Neutro	7,5	Neutro	7,5	Neutro		
pH (KCl)									
Condutividade Eléct.	mS cm^{-1}								
Necessidade em «cal»	t/ha $CaCO_3$*	0,0		0,0		0,0			
Fósforo extraível	mg P_2O_5 kg^{-1}	330	Muito alta	261	Muito alta	382	Muito alta		
Potássio extraível	mg K_2O kg^{-1}	356	Muito alta	202	Muito alta	381	Muito alta		
Magnésio extraível	mg Mg kg^{-1}								

Annex V- Biometric parameters associated with the ear - average data from Organic Vs Conventional trial (2020)

Genotype	L	ED1	ED3	ED2	ED4	R1	R2
2444 - (Cald 19)	15,1	4,9	4,7	4,5	4,3	9	8,9
2448 - (Cald 18)	15	5	4,8	4,6	4,4	11,5	11,5
2449 - (Cald 19)	14,2	5,2	5	4,6	4,5	12,5	12,3
2488 - (Cald 18)	13,6	5,2	5	4,7	4,5	12,7	12,6
2489 - (Cald 18)	14,8	4,9	4,6	4,3	4,2	9,7	9,6
2493 - (Cald 18)	15	4,9	4,7	4,3	4,1	12,4	12,1
2494 - (Cald 18)	14,6	5	4,8	4,4	4,3	11,9	11,7
2496 - (Cald 18)	16,1	5,2	5	4,7	4,5	10,7	10,6
2498 - (Cald 19)	14,3	4,8	4,7	4,4	4,2	12	11,9
2499 - (Cald 19)	16,1	5,1	5	4,6	4,4	11,6	11,6
2501 - (Cald 19)	14,2	5,4	5,2	4,9	5,8	12,8	12,5
2502 - (Cald 19)	14,8	4,9	4,7	4,4	4,3	11,1	11,1
2504 - (Cald 19)	13,7	5,4	5,1	5	4,8	13,5	13,4
2505 - (Cald 18)	13	5,3	5,1	4,8	4,7	14	13,6
2507 - (Cald 19)	14,6	5,2	4,9	4,6	4,5	13	12,7
2508 - (Cald 19)	14	5	4,8	4,5	4,3	13	12,6
2509 - (Cald 18)	14	5	4,7	4,5	4,3	10,9	12,5
2510 - (Cald 19)	14,7	5,4	5,2	4,8	4,7	13,8	13,5
2513 - (Cald 19)	13,9	5,1	4,9	4,6	4,5	12	11,7
2514 - (Cald 19)	14,7	5,1	4,9	4,6	4,4	13	12,7
2515 - (Cald 18)	16,3	5,4	5,2	4,8	4,6	13,5	13,5
2516 - (Cald 19)	14,7	5,5	5,3	4,7	4,5	14,1	13,7
2517 - (Cald 19)	13,8	5,4	5,2	4,9	4,8	13	12,8
2518 - (Cald 18)	14,9	5,1	5	4,6	4,5	12,3	12,2
2519 - (Cald 19)	14,3	4,9	4,6	4,4	4,2	12,1	11,9
2522 - (Cald 19)	13,8	5,3	5,1	4,7	4,5	12,4	12
2524 - (Cald 18)	14,8	5,3	5,1	4,8	4,7	13,1	13
2525 - (Cald 19)	14,2	4,9	4,8	4,3	4,2	10,5	10,4
2526 - (Cald 19)	16,9	5,3	5,1	4,7	4,5	9,9	9,6
2527 - (Cald 18)	15,9	5,2	5	4,8	4,7	10,8	10,7
2528 - (Cald 18)	16,1	5,2	5,1	4,6	5,2	12,1	11,9
2529 - (Cald 19)	13,7	5,1	4,9	4,6	4,5	13	12,8
2530 - (Cald 19)	16,2	5	5	4,5	4,4	12	11,9
2531 - (Cald 19)	15,1	4,9	4,6	4,3	4,2	12,2	12,1
BSM17 - (Cald 18)	13,3	4,8	4,6	4,2	4,1	12	11,8
BT17 - (Cald 18)	14,1	5,4	5,1	5	4,8	11,1	10,8
BT18 - (Cald 19)	14,3	5,3	5,1	4,8	4,6	11,4	11,4
Bulk-Azores 2 - (Cald 19)	15,8	5,3	5,1	4,7	4,6	12,2	12,1
Bulk-Azores1 - (Cald 18)	15,9	5,2	5	4,7	4,6	12	12
MONJ-2 - (Cald 18)	14,2	4,9	4,6	4,2	4,1	12,7	12,5
MONJ-3 - (Cald 18)	18,3	4,9	4,8	4,4	4,3	12,6	12,4
MT17 - (Cald 18)	15,5	5,4	5,2	5	4,8	11,2	11,1
Pg 18 - (Cald 18)	14,2	6,5	5,2	5,2	4,7	20,4	20,3
Pg 19 - (Cald 19)	15,4	6	5,6	5,4	4,9	21,2	20,2
Pg 19 - (Lou 19)	14,2	5,8	5,4	5,2	4,9	19,6	19,2
SinPre - (Cald 19)	16,8	4,8	4,6	4,2	4	16,1	15,3
VA 17 - (Seq Lou 17)	15,8	4,7	4,5	4,2	4	13,5	13,6
VA 19 - (Reg Lou 19)	16	4,8	4,6	4,2	4,1	13,7	13,5
VA 19 - (Seq Lou 19)	15,7	4,5	4,3	3,9	3,8	13,7	13,6
VT17 - (Cald 18)	14,6	5,5	5,3	5	4,8	11,2	10,9

Annex VI- Biometric parameters associated with the ear - average data from Organic Vs Conventional trial 2020 (Averages)

Genotype	Fa	D/I	TR	CV	F/D	KC	EW
2444 - (Cald 19)	1,5	1	1,5	1,6	7	3	157,9
2448 - (Cald 18)	1,8	1	1,3	1,8	7	3	158,3
2449 - (Cald 19)	1,5	1,2	1,4	1,8	6	3,3	149,9
2488 - (Cald 18)	2,2	1	1,1	1,5	7	2,8	157,2
2489 - (Cald 18)	1,3	1	1,3	1,7	7	3	142,5
2493 - (Cald 18)	1,6	1	1,3	2,1	6	5	155,6
2494 - (Cald 18)	1,9	1	1,4	1,8	7	3	175,4
2496 - (Cald 18)	1,6	1	1,4	1,6	7	3	179,7
2498 - (Cald 19)	1,8	1	1,5	1,7	7	3	142,7
2499 - (Cald 19)	1,3	1	1,3	1,4	7	5,5	173,9
2501 - (Cald 19)	1,6	1	1,3	1,9	7	3	155,8
2502 - (Cald 19)	1,7	1	1,3	1,4	7	3	142,7
2504 - (Cald 19)	1,8	1	1,4	1,7	7	3	159,3
2505 - (Cald 18)	2,1	1	1,6	1,9	6	3,5	129,7
2507 - (Cald 19)	2,1	1	1,6	1,9	7	2,8	140,6
2508 - (Cald 19)	1,6	1	1,1	1,8	7	3,3	139
2509 - (Cald 18)	2,1	1	1,2	1,8	7	3,3	141,3
2510 - (Cald 19)	1,8	1	1,5	1,9	7	3	181
2513 - (Cald 19)	1,6	1	1,3	1,4	7	3	149,1
2514 - (Cald 19)	1,7	1	1,2	1,5	7	3,3	143,3
2515 - (Cald 18)	2,1	1	1,2	1,4	7	3	171,4
2516 - (Cald 19)	1,8	1	1,5	2,2	6	3,3	157,9
2517 - (Cald 19)	2,2	1	1,5	2	7	3	154,1
2518 - (Cald 18)	1,5	1	1,4	1,9	7	3,2	166,2
2519 - (Cald 19)	3,2	1	1,4	1,6	6	3,3	134
2522 - (Cald 19)	1,9	1	1,1	2,2	7	3	164,4
2524 - (Cald 18)	1,7	1	1,4	1,5	7	3	169,8
2525 - (Cald 19)	1,5	1	1,5	1,9	7	3	132,8
2526 - (Cald 19)	1,4	1	1,2	2,1	7	3	183,8
2527 - (Cald 18)	1,8	1	1,1	1,5	7	3	175,5
2528 - (Cald 18)	1,7	1	1,5	2	6	3,4	179,7
2529 - (Cald 19)	1,8	1	1,2	1,7	6	3	143,4
2530 - (Cald 19)	1,4	1	1,1	1,3	7	3	158
2531 - (Cald 19)	1,6	1	1,3	1,7	7	2,8	154,3
BSM17 - (Cald 18)	1,8	1	1,3	1,6	7	3	129,6
BT17 - (Cald 18)	1,8	1	1,3	1,9	7	3	152
BT18 - (Cald 19)	1,8	1	1,4	1,5	7	3	149,4
Bulk-Azores 2 - (Cald 19)	1,6	1	1,3	1,7	7	5	176,6
Bulk-Azores1 - (Cald 18)	1,8	1	1,2	1,3	7	3	177,9
MONJ-2 - (Cald 18)	1,8	1	1,3	1,7	6	4,3	121,3
MONJ-3 - (Cald 18)	1,4	1	1,2	1,5	6	9	144,3
MT17 - (Cald 18)	2,1	1	1,5	1,7	7	5,8	169,5
Pg 18 - (Cald 18)	3,4	1	1,5	2,7	6	3,7	133,5
Pg 19 - (Cald 19)	2,7	1	1,6	2,6	5	3,8	181,5
Pg 19 - (Lou 19)	2,4	1	1,4	2,1	6	3,4	170,3
SinPre - (Cald 19)	1,6	1	1,3	1,6	5	3,3	139
VA 17 - (Seq Lou 17)	1,7	1	1,4	1,6	6	3	140,2
VA 19 - (Reg Lou 19)	1,4	1	1,2	1,4	6	3	149
VA 19 - (Seq Lou 19)	1,5	1	1,4	1,4	6	3,7	136,1
VT17 - (Cald 18)	1,5	1	1,4	2,1	7	5,5	149,4

Annex VII- Biometric parameters associated with the ear - average data from Organic Vs Conventional trial 2020 (Averages)

Genotype	CW	KW15	KD	SW	HR	NC	DC1	DC3
2444 - (Cald 19)	24,8	137	0,4	510,8	12,2	29,3	2,8	2,5
2448 - (Cald 18)	24,5	138,6	0,5	432,7	11,5	27,8	2,9	2,6
2449 - (Cald 19)	24	129,5	0,4	400,5	12,2	26,6	3,4	2,8
2488 - (Cald 18)	28,8	132,8	0,5	404,6	11,7	25,4	3,1	2,9
2489 - (Cald 18)	21,2	126,5	0,4	433,9	11,1	28,6	2,5	2,4
2493 - (Cald 18)	26,3	110,2	0,4	365,5	12,3	28,2	3,1	2,9
2494 - (Cald 18)	25,1	154,3	0,5	399,2	12,3	27,7	3	2,9
2496 - (Cald 18)	28,7	156,1	0,4	520,2	11,8	28,2	3	2,8
2498 - (Cald 19)	21,3	125,2	0,4	381	12,3	26,8	2,9	2,7
2499 - (Cald 19)	29,9	148,9	0,4	413,6	11,8	30,5	3	2,7
2501 - (Cald 19)	29,7	131,4	0,5	408,9	11,3	25,7	3,3	3
2502 - (Cald 19)	23,1	124,8	0,5	458	11,2	26,2	2,9	2,7
2504 - (Cald 19)	29	135,5	0,4	432	11,2	26	3,1	2,9
2505 - (Cald 18)	21,8	112	0,5	318,7	11,4	23,5	3,1	2,9
2507 - (Cald 19)	22,4	123,1	0,5	417,8	11,4	27,2	3,1	2,8
2508 - (Cald 19)	25,4	116,4	0,5	380,7	12	26,5	3	2,7
2509 - (Cald 18)	25,1	120,5	0,5	420,8	10,9	23	3	2,8
2510 - (Cald 19)	27,1	160,5	0,4	401,5	11,3	27,9	3,2	2,9
2513 - (Cald 19)	22,3	131,7	0,4	442	11,3	25	2,8	2,6
2514 - (Cald 19)	25	121,8	0,5	370,2	12	26,8	3,1	2,8
2515 - (Cald 18)	33,2	142,2	0,4	367,2	12,1	27,2	3,5	3,2
2516 - (Cald 19)	28	133,2	0,4	366,3	12,6	26,4	3,6	3,3
2517 - (Cald 19)	27,6	131,1	0,5	454,8	11,5	22,3	3,8	3
2518 - (Cald 18)	27,4	141,4	0,5	419,6	12,7	27,6	3	2,9
2519 - (Cald 19)	24,3	114	0,4	392,8	11,1	24,2	9,3	2,8
2522 - (Cald 19)	25,2	143,6	0,4	400,8	12	27,2	3,1	2,8
2524 - (Cald 18)	28,3	144,8	0,4	432,3	12,5	26,3	3,2	2,8
2525 - (Cald 19)	22,4	114,7	0,5	435,1	11,3	24,7	2,9	8,9
2526 - (Cald 19)	37,3	152,1	0,4	476	11,4	30,7	3,2	3
2527 - (Cald 18)	28,8	149,8	0,5	503,8	12,8	27,6	3	2,8
2528 - (Cald 18)	30	152,4	0,5	427,2	12,9	29,8	3,1	2,9
2529 - (Cald 19)	23,5	124,2	0,5	423	11,7	24,8	3,1	2,8
2530 - (Cald 19)	28,7	131,9	0,5	421,6	13,4	29	3,1	3
2531 - (Cald 19)	25,8	131,7	0,5	406,3	12,2	26,5	3	2,7
BSM17 - (Cald 18)	20,8	112,3	0,5	399,2	11,8	23,7	3	2,7
BT17 - (Cald 18)	31,5	125,4	0,4	478,3	10,6	24,8	3,3	3
BT18 - (Cald 19)	27,2	128,3	0,5	432,4	10,4	26,8	3,2	3
Bulk-Azores 2 - (Cald 19)	27,6	154,1	0,5	427,7	12,3	29,5	3,1	2,9
Bulk-Azores1 - (Cald 18)	28,6	153,3	0,4	429,7	12,2	29,6	3,2	3
MONJ-2 - (Cald 18)	23,2	101,5	0,5	319,5	11,4	25,4	3,2	2,9
MONJ-3 - (Cald 18)	23,8	126,6	0,5	382	10,2	27,7	3	2,7
MT17 - (Cald 18)	36,3	137,7	0,5	495,2	11,6	25	3,6	3,3
Pg 18 - (Cald 18)	25,8	111,1	0,5	246	11,5	21,9	4,1	3,5
Pg 19 - (Cald 19)	33,5	152,7	1,4	280	12,3	27,2	4,3	3,9
Pg 19 - (Lou 19)	32,8	140,6	0,5	316	12,6	25,6	4,1	3,8
SinPre - (Cald 19)	24,2	115,1	0,5	283,3	13,6	30,7	3,3	3
VA 17 - (Seq Lou 17)	22	120,5	0,4	317,3	12,9	28,5	3,1	2,8
VA 19 - (Reg Lou 19)	22,6	129,6	0,4	323,7	12,9	30,8	3,1	2,8
VA 19 - (Seq Lou 19)	21,2	117,9	0,4	305,3	12,5	28,9	3	2,8
VT17 - (Cald 18)	38,8	121,6	0,5	486	11,3	23,3	3,5	3,3

Annex VIII- Biometric parameters associated with the ear - average data from Organic Vs Conventional trial 2020 (Averages)

Genotype	DC2	DC4	M1	M2	Rq1	Rq2	CC
2444 - (Cald 19)	2,3	2,1	0,9	0,7	1,7	1,5	2
2448 - (Cald 18)	2,4	2,2	1	0,9	1,8	1,6	2
2449 - (Cald 19)	2,5	2,3	0,9	0,8	1,8	1,6	1
2488 - (Cald 18)	2,7	2,5	1,1	2,3	2	1,8	2
2489 - (Cald 18)	2,1	2	0,8	0,7	4,2	1,3	1
2493 - (Cald 18)	2,5	2,4	1	0,9	1,9	1,8	2
2494 - (Cald 18)	2,5	2,4	1	0,8	1,8	1,7	2
2496 - (Cald 18)	2,5	2,3	0,8	0,7	1,6	1,5	1
2498 - (Cald 19)	2,5	2,4	0,9	0,9	6,7	1,7	1
2499 - (Cald 19)	2,4	2,2	1	0,9	1,7	1,5	1
2501 - (Cald 19)	2,6	2,4	1,1	1	2	1,9	2
2502 - (Cald 19)	2,4	2,3	0,9	0,8	1,8	1,6	2
2504 - (Cald 19)	2,5	2,6	0,9	0,8	1,7	1,5	2
2505 - (Cald 18)	2,7	2,5	1,2	2,6	2	1,8	2
2507 - (Cald 19)	2,6	2,5	1,1	1	2	1,9	2
2508 - (Cald 19)	2,5	2,3	1	0,8	1,9	1,7	2
2509 - (Cald 18)	2,6	2,5	1	1,1	2	1,8	2
2510 - (Cald 19)	2,6	2,5	1	0,8	1,8	1,6	2
2513 - (Cald 19)	2,4	2,2	0,9	0,8	1,5	1,3	2
2514 - (Cald 19)	2,7	2,4	1	0,9	4,5	1,7	2
2515 - (Cald 18)	2,9	2,7	1,2	1,5	2,2	2	2
2516 - (Cald 19)	3	2,8	1,2	1	2,2	1,9	2
2517 - (Cald 19)	2,8	2,6	1,1	1	2,3	2	1
2518 - (Cald 18)	2,6	2,4	1,1	1	1,9	1,7	2
2519 - (Cald 19)	2,5	2,4	1	0,9	1,9	1,9	1
2522 - (Cald 19)	2,5	2,3	1	0,9	1,9	1,7	2
2524 - (Cald 18)	2,6	2,4	1	0,8	1,8	1,6	2
2525 - (Cald 19)	2,4	2,2	1	0,9	1,7	1,6	2
2526 - (Cald 19)	2,5	2,4	1	0,9	2,2	2	2
2527 - (Cald 18)	2,5	2,3	1	0,9	2	1,8	2
2528 - (Cald 18)	2,5	2,4	1,1	1	2	1,9	2
2529 - (Cald 19)	2,8	2,3	1	0,9	1,9	1,7	1
2530 - (Cald 19)	2,5	2,4	0,9	0,8	1,8	1,7	2
2531 - (Cald 19)	2,7	2,1	1	0,8	1,8	1,6	2
BSM17 - (Cald 18)	2,4	2,3	1,1	0,8	1,4	1,4	2
BT17 - (Cald 18)	2,8	2,5	1	0,9	1,9	1,7	2
BT18 - (Cald 19)	2,5	2,4	1,1	1,1	1,9	1,8	1
Bulk-Azores 2 - (Cald 19)	2,6	2,4	1	0,9	1,9	1,7	2
Bulk-Azores1 - (Cald 18)	2,7	2,6	1,1	1	2	1,8	2
MONJ-2 - (Cald 18)	2,6	2,5	1	0,9	2	1,8	2
MONJ-3 - (Cald 18)	2,2	2,1	0,9	0,8	1,6	1,5	2
MT17 - (Cald 18)	3	2,8	1,1	1	2,1	1,9	1
Pg 18 - (Cald 18)	3,6	3	1,9	1,3	2,9	2,2	2
Pg 19 - (Cald 19)	3,8	3,1	1,9	1,3	3,1	2,3	2
Pg 19 - (Lou 19)	3,4	3	1,7	1,2	2,8	2,2	2
SinPre - (Cald 19)	2,6	2,4	1,1	1	2,1	1,8	2
VA 17 - (Seq Lou 17)	2,6	2,4	1	0,9	2,1	1,8	2
VA 19 - (Reg Lou 19)	2,4	2,3	0,9	0,8	1,7	1,6	2
VA 19 - (Seq Lou 19)	2,5	2,4	0,9	0,8	1,9	1,7	2
VT17 - (Cald 18)	2,9	2,7	1,1	0,9	5,2	1,7	1

Annex IX- Biometric parameters associated with the ear - average data from On-Farm PPB trial 2020 (Averages)

Genotype	L	ED1	ED3	ED2	ED4	R1	R2
Amc397 - (Lou 19)	18,9	4,7	4,4	3,9	3,7	14,9	15,1
Bulk-Azores 2 - (Cald 19)	17,5	5,6	5,4	4,9	4,7	12,2	12,2
Fn 2014 - (Lou 19)	21	5,7	5,3	5,3	4,9	20,1	20,1
Pg 18 - (Cald 18)	15,3	6,1	5,6	5,6	7,2	24	23,8
Pg 19 - (Cald 19)	15,5	5,9	5,4	5,5	5	21,8	21,7
Pg 19 - (Lou 19)	14,8	5,8	5,3	5,2	4,6	22,7	22,4
SinPre - (Cald 19)	18,1	4,8	4,6	4	3,7	16	16
VA 17 - (Seq Lou 17)	16,9	4,5	4,3	4	3,7	13,7	13,6
VA 19 - (Reg Lou 19)	17,8	4,6	4,3	3,9	3,8	14,1	14
VA 19 - (Seq Lou 19)	17,8	4,4	4,2	3,8	3,6	13,5	13,5

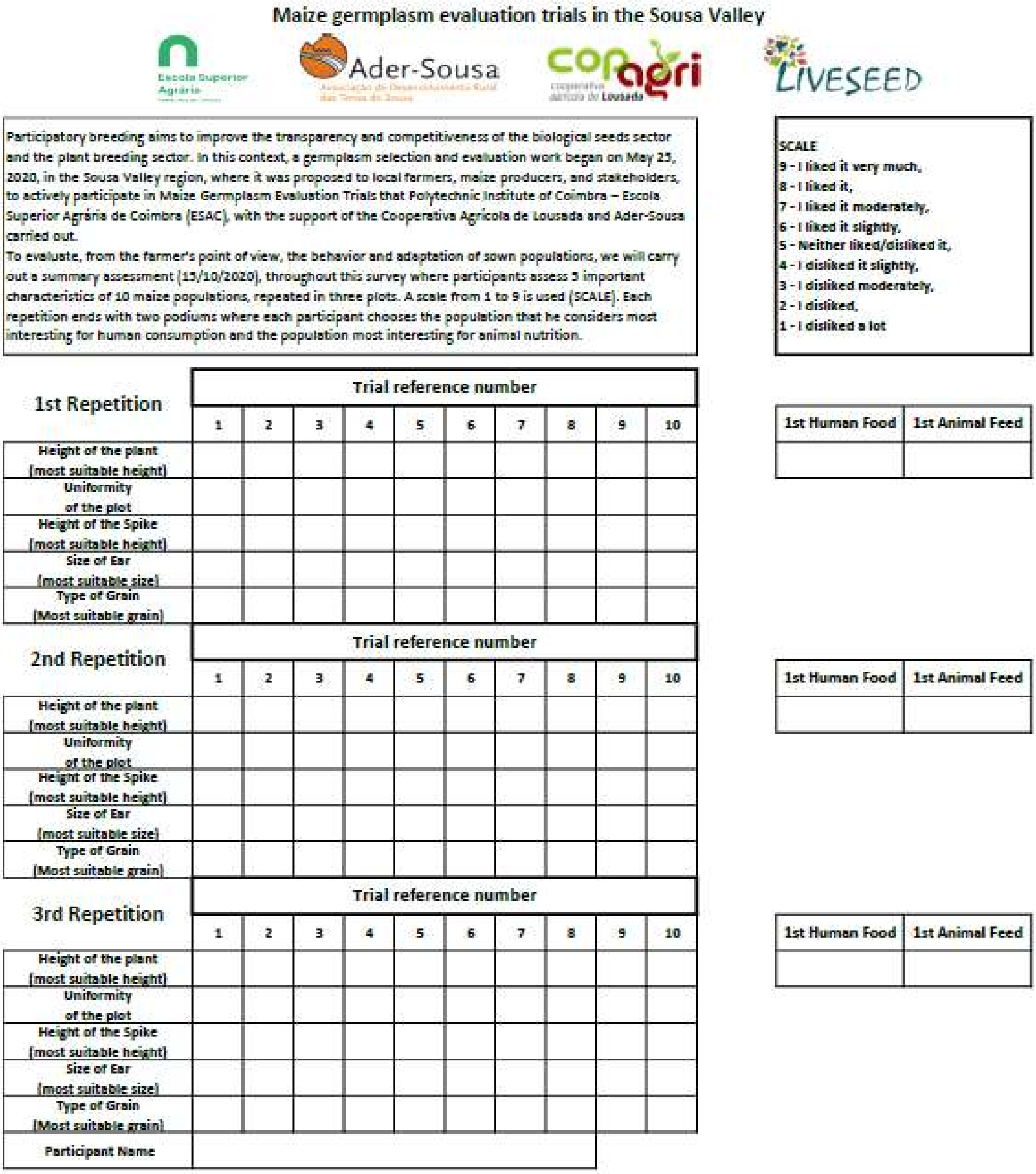
Genotype	Fa	D/I	TR	CV	F/D	KC	EW
Amc397 - (Lou 19)	1,4	1	1,4	1,8	3	4,7	167
Bulk-Azores 2 - (Cald 19)	1,3	1	1	1,9	7	5,3	214,5
Fn 2014 - (Lou 19)	1,5	1	1,4	1,8	6	3,3	271,8
Pg 18 - (Cald 18)	4,1	2	1,5	2,9	4	3	193,2
Pg 19 - (185-2019 Cald 19)	3,3	1	1,2	2,6	4	3,4	191,3
Pg 19 - (Lou 19)	3,8	2	1,2	2,7	4	3	178,6
SinPre - (Cald 19)	1,7	1	1,3	1,9	4	4	157,5
VA 17 - (Seq Lou 17)	1,6	1	1,1	1,7	5	3	146,4
VA 19 - (Reg Lou 19)	1,9	2	1,1	1,5	4	3	172,4
VA 19 - (Seq Lou 19)	2	2	1,3	1,8	5	3	161,5

Genotype	CW	KW15	KD	SW	HR	NC
Amc397 - (Lou 19)	24,1	141,5	0,5	324,8	15,8	34,4
Bulk-Azores 2 - (Cald 19)	104,1	196,7	0,5	413,5	15,6	33,1
Fn 2014 - (Lou 19)	58	205,2	0,5	340,4	18,1	41,1
Pg 18 - (Cald 18)	36,3	155	0,5	336,7	16	29,3
Pg 19 - (185-2019 Cald 19)	38,7	150,5	0,5	301,4	16	28
Pg 19 - (Lou 19)	35,5	141,1	0,5	334,3	16,1	26,8
SinPre - (Cald 19)	26,5	130,1	0,5	348,7	15,5	32,8
VA 17 - (Seq Lou 17)	22,9	122,4	0,5	325,3	15,7	32,5
VA 19 - (Reg Lou 19)	25,6	146	0,5	337,7	15,5	34,6
VA 19 - (Seq Lou 19)	23,9	137	0,5	332,2	15,3	33,7

Annex X- Biometric parameters associated with the ear - average data from On-Farm PPB trial 2020 (Averages)

Genotype	DC1	DC3	DC2	DC4	M1	M2	Rq1	Rq2	CC
Amc397 - (Lou 19)	3,2	2,9	2,3	2,1	2,1	1,8	2	4	2
Bulk-Azores 2 - (Cald 19)	3,3	3,1	2,4	2,2	1,2	1	2,1	1,9	2
Fn 2014 - (Lou 19)	3,6	3,1	2,9	2,5	1,6	1,2	2,6	2,2	1
Pg 18 - (Cald 18)	4,6	3,9	3,7	3,1	1,8	1,3	2,4	2	2
Pg 19 - (Cald 19)	4,4	3,8	3,8	2,9	2,1	1,3	2,8	2,2	2
Pg 19 - (Lou 19)	4,3	3,7	3,5	2,9	1,7	1,3	2,4	2	2
SinPre - (Cald 19)	3,6	3,3	2,7	2,4	2,9	2,4	5,1	4,2	2
VA 17 - (Seq Lou 17)	3,1	2,8	2,3	2,1	2,5	1,9	3,7	3	2
VA 19 - (Reg Lou 19)	3,1	2,8	2,3	2,1	2,2	2	3,5	3	2
VA 19 - (Seq Lou 19)	3	2,8	2,2	2	2,1	1,8	3,6	3,2	2

Annex XI- Survey of Maize germplasm evaluation trials in the Sousa Valley (2020)



Maize germplasm evaluation trials in the Sousa Valley

Participatory breeding aims to improve the transparency and competitiveness of the biological seeds sector and the plant breeding sector. In this context, a germplasm selection and evaluation work began on May 25, 2020, in the Sousa Valley region, where it was proposed to local farmers, maize producers, and stakeholders, to actively participate in Maize Germplasm Evaluation Trials that Polytechnic Institute of Coimbra – Escola Superior Agrária de Coimbra (ESAC), with the support of the Cooperativa Agrícola de Lousada and Ader-Sousa carried out.
To evaluate, from the farmer's point of view, the behavior and adaptation of sown populations, we will carry out a summary assessment (15/10/2020), throughout this survey where participants assess 5 important characteristics of 10 maize populations, repeated in three plots. A scale from 1 to 9 is used (SCALE). Each repetition ends with two podiums where each participant chooses the population that he considers most interesting for human consumption and the population most interesting for animal nutrition.

SCALE
9 - I liked it very much,
8 - I liked it,
7 - I liked it moderately,
6 - I liked it slightly,
5 - Neither liked/disliked it,
4 - I disliked it slightly,
3 - I disliked moderately,
2 - I disliked,
1 - I disliked a lot

1st Repetition	Trial reference number									
	1	2	3	4	5	6	7	8	9	10
Height of the plant (most suitable height)										
Uniformity of the plot										
Height of the Spike (most suitable height)										
Size of Ear (most suitable size)										
Type of Grain (Most suitable grain)										

1st Human Food	1st Animal Feed

2nd Repetition	Trial reference number									
	1	2	3	4	5	6	7	8	9	10
Height of the plant (most suitable height)										
Uniformity of the plot										
Height of the Spike (most suitable height)										
Size of Ear (most suitable size)										
Type of Grain (Most suitable grain)										

1st Human Food	1st Animal Feed

3rd Repetition	Trial reference number									
	1	2	3	4	5	6	7	8	9	10
Height of the plant (most suitable height)										
Uniformity of the plot										
Height of the Spike (most suitable height)										
Size of Ear (most suitable size)										
Type of Grain (Most suitable grain)										

1st Human Food	1st Animal Feed

Participant Name