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Investigating Germplasm Collection and Development Potential of Organic Cowpea in Portugal

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Undersökning av frösamling samt utvecklingspotential av ekologiska ögonbönor i Portugal

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Abstract

Initial trials were conducted in Idanha-a-Nova, Portugal together with a literature review with the aim to investigate the development potential of an organic plant breeding and seed company's (Sementes Vivas) cowpea germplasm collection in relation to organic agriculture, sustainable development, and plant breeding. This work discusses different development directions such as intercropping, sole cropping and home gardens as well as organic plant breeding of the cowpea crop. Different cultivation methods require cultivars with different plant characteristics to perform at an optimum level. Traits that were investigated are; seed length, seed width, seed thickness, 100-seed weight, seed coating colour, testa (seed coat) texture, seed eye pattern, seed eye colour, growth habit, plant vigour, twinning tendency, terminal leaflet length, terminal leaflet width, terminal leaf let shape, leaf colour, petiole length, petiolule length, length of main stem, number of main branches, days to flowering, flower colour, immature pod pigmentation. The germplasm collection turned out to contain genetic variation that might be useful for the different development directions although there might be benefits of gathering more genetic material and doing more research in order to conduct well-planned and cost efficient development of cowpea cropping systems and breeding programs.

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Abbreviations

Acc.	accession
°C	degree Celsius
cm	centimetre
CO_2	carbon dioxide
e.g.	example given
g	gram
l	litre
LER	land equivalent ratio
LSD	least significant difference
mg	milligram
mm	millimetre
ssp.	subspecies

Introduction & Background

Legumes from a sustainability perspective

Many of the benefits of cultivating cowpea lies within the spectrum of benefits associated to legumes in general. There are several aspects of legume cropping and consumption that could contribute to a more sustainable agricultural practise but also human life style in general. Stagnari F. *et al.* (2017) mentions the points:

- Lower emissions of greenhouse gases, carbon dioxide, and nitrous oxide compared to agricultural systems based on mineral N-fertilization.
- Importance for the sequestration of carbon in soils.
- Reduction of the overall fossil energy inputs in the system.

The same article also includes information about agronomic pre-crop benefits such as the 'nitrogen effect' which is the N provision coming from the legumes ability to biologically fix nitrogen which is most efficient when the N-fertilization has been low in the subsequent crop cycles. The other benefit is the 'break crop effect' involves aspects not necessarily related to legumes but rather general crop rotation benefits of which the legume crop can contribute with as well. These aspects are improvement of soil organic matter and structure, phosphorus mobilization, soil water retention and availability, and reduced pressure from pests, diseases, and weeds (Stagnari F.*et al.,* 2017).

Legumes do not only have the potential to contribute to sustainability through agricultural aspects but can also make a difference based on human life style changes related to our consumption. A multidisciplinary study conducted on Dutch consumers concluded that if the consumers were to reduce their protein intake by a third (based on an observation that Dutch consumers in average consumes 60 % more protein than the RDI (recommended daily intake) and replace their overall protein intake by either plant derived proteins or extensively produced meat, a substantial reduction of the pressure on the environment could be obtained without putting a healthy nutrition at risk (Aiking, H. *et al.,* 2006). Legumes with high protein content could play an important role for the substitution of meat proposed in this study. (Multari S. *et al.,* 2015) concludes that alternative protein sources produced in a sustainable manner are required to feed the worlds growing population. They also state that legumes (in this case fava bean) partially can replace meat and meat based products in the human diet.

Actors involved in the case study

Sementes Vivas

Sementes Vivas is a new started organic plant breeding and seed producing company located in Idanha-a-Nova, Portugal. Their main breeding objectives are to produce varieties well adapted to organic and biodynamic farming conditions, alternative farming practises (e.g. intercropping) and crop varieties adapted to climate change (e.g. drought and heat tolerance).

INIAV

INIAV is the agricultural gene bank of Portugal. They have been contributing with traditional Portuguese cultivars to the trial and there are on-going plans for a potential future collaboration between Sementes Vivas and INIAV to work towards sustainable and climate change adapted cowpea intercropping systems.

The cowpea project and the initial trials

This thesis work is part of an initiative by Sementes Vivas together with INIAV to investigate and develop cowpea cultivation in Portugal and will be referred to as 'the cowpea project' in this report. The long and short term ambitions and objectives of the cowpea project have not clearly stated during the time this thesis have been conducted. The work has been taking place during the very initial stage of the project which includes the investigation of what is reasonable to include or not. In a broad perspective the aim is to figure out a way to include and develop cowpea for future organic and biodynamic agricultural practises in Portugal and its surrounding regions. It can also be seen as a part of an even bigger initiative to investigate crops and cultivation methods for climate change adaptation and mitigation. The first year (2016) includes the initial sole crop trials which was the ground work for this thesis and a smaller trial that was sown earlier and conducted by an employee at Sementes Vivas. It is not yet certain how the project will proceed but during the time the trials were conducted the plan was to perform intercropping trials with maize the upcoming years since this part (intercropping) of the cowpea project has been granted funding from the EU Research and Innovation programme Horizon 2020. Hopefully the data collection and literature review of this thesis can help as an information base to make decisions regarding the future of the project. The general objectives discussed in the company during the first year have been:

- Develop a cowpea intercropping practise with maize (and potentially other crops).
- Find or breed cowpea cultivars adapted to organic intercropping and sole cropping systems as well as local environmental conditions.
- Select or breed cultivars suited for home gardens.

It should also be mentioned that the budget for the project is very limited. This thesis work does not involve any qualified cost or profit calculations although the financial limitations have been considered when suggesting different methods and sizes of future trials.

Cowpea introduction

Cowpea (*Vigna unguiculata* L.Walp) belongs to the family Fabaceae. It is diploid with 2*n* = 2*x* = 22 chromosomes (Faris D.G., 1964) and highly self-pollinated (Williams C.B. and Chambliss O.L., 1980). Cowpea has many different names worldwide. Other typical names in English are Southernpea, Blackeyed peas, and Field peas (Filho, F.R.F. *et al.,* 2012). In Portugal they are mainly called Feijäo-Frade and sometimes also Feijäo-Fradinho while Brazilians call them Feijäo-Caupi (personal observation, 2016). The name cowpea most commonly refers to the ssp. (subspecies) *unguiculata* (*Vigna unguiculata* ssp. *unguiculata)*. Boudoin J.P. and Maréchal R. (1985) mentions four cultivar groups: *unguiculata* which is the common type, *biflora* (previously *catjang)* which has small erect pods and grows in Asia, *sesquipedalis* which also mainly grows in Asia and has very long pods that are consumed as vegetables, and *textilis* that has been grown in West Africa for fibre production. This thesis mainly focuses on the *unguiculata* group but also *sesquipedalis* cultivars have been included in the trial. The origin of the species is disputed but a wide range of diversity has been found for the wild subspecies in southeast Africa (Ng N.Q. and Maréchal R., 1985). It is especially valuable as food due to its high protein content (20 – 27 %) in the dried grains (Kochhar, N. & Walker, A.F., 1986). In Portugal the dried grains are used to prepare various dishes (personal observation, 2016) which also is the most common usage in most of the world although some countries also use other parts of the plant for consumption (Madodé Y.E., *et al.,* 2011). All parts of the plant can also be used as animal fodder (Singh B.B. *et al.,* 1997).

Filho F.R.F. *et al.* (2012) summarize the cowpea production levels of the world for the period 2005 to 2009 and concludes that Nigeria was the top producer followed by Niger, Brazil, and Burkina Faso. The same articles also list the European cowpea producing countries; Portugal, Turkey, Greece, Italy, Bulgaria, Spain, Serbia, Croatia, Macedonia Republic, Bosnia and Herzegovina, and Hungary. The European cowpea production only represents a small fraction of the global production. The total European cowpea production estimates an average of 23 659 tonnes over the mentioned period compared to 2 788 166 tonnes by Nigeria alone or 505 233 tonnes by Brazil. Portugal is also listed to be one of the cowpea importing countries which also can be seen in the supermarkets where the origin of the cowpea usually is Peru or Brazil (personal observation, 2016).

Consumer preferences

Cowpea has a wide spectrum of appearance, textures and cooking qualities. Although people from Europe and perhaps most of the western world probably associate cowpea with the typical black eye look (white bean with a black ring around the hilum) there are plenty of other types of cowpea consumed around the world. For instance; people in West and Central Africa prefer seeds with rough tegument texture (because they remove the tegument when they cook traditional dishes) while in Eastern and Southern Africa people prefer smooth tegument texture instead (Singh B.B. and Ishiyaku M.F., 2000). In Portugal the main type of cowpea available is the typical black eye that is imported and sold in the supermarkets (personal observation, 2016). There are also local varieties in Portugal differentiating from the typical black eyed one (personal observation, 2016). Since at least some of these native varieties are being sold in local markets and smaller shops (to a premium price - see figure 1) around the country there still has to be some demand for "alternative" cowpea varieties in Portugal. It is also possible to find bags with cowpea that has heterogeneous appearances in farmers markets (see figure 2).

Figure 1. Price comparison between domestic cowpea variety 'Arroz' 6,49 euro/l (left) and imported typical black eyed type 1,40 euro/l (right).

Figure 2. Differences in seed appearance within the same bag (found at a local farmers market in São Pedro do Sul).

Organic farming systems

To understand the importance of selection or breeding for specific plant traits and chose appropriate crop management methods for organic farming conditions it is good to know the difference between conventional and organic farming systems. The USDA describes organic agriculture as (USDA, 2007):

"… an ecological production management system that promotes and enhances biodiversity, biological cycles, and soil biological activity. It is based on a minimal use of off-farm inputs and on management practices that restore, maintain, and enhance ecological harmony."

The IFOAM defines organic agriculture as (IFOAM, 2005):

"Organic Agriculture is a production system that sustains the health of soils, ecosystems and people. It relies on ecological processes, biodiversity and cycles adapted to local conditions, rather than the use of inputs with adverse effects. Organic Agriculture combines tradition, innovation and science to benefit the shared environment and promote fair relationships and a good quality of life for all involved."

In practise this means that organic farming systems has less options when it comes to managing external threats to the production such as weeds and diseases. The following (until end of section) aspects that distinguish organic farming systems from conventional ones are mentioned in an article by Lammerts van Bueren E.T. and Myers R. (2012); In conventional systems it is possible to apply synthetic-chemical crop protectants (herbicides, pesticides, and fungicides) while organic systems rely on a few organically approved inputs, longer crop rotations (6 to 10 years), mechanical weeding, no tillage systems with mulching, stale seedbeds, and the use of crop competition. Organic regulations also do not allow chemicals to be used for seed treatment and sprouting inhibition, instead it can use hot water or steaming or a few options of organic additives (e.g. mustard powder). Organic farmers can only use lower (compared to conventional) levels of fertilizers and they have to be organic. The sources can be animal manure, compost, and green manures which give a slower release of nutrients and have to be managed with a more long term perspective compared to conventional mineral nutrients. Biodiversity is also an important factor of organic farming systems; both as a product of the system itself and as a tool for keeping the system resilient. The biodiversity includes beneficial soil organisms, crop species and varietal diversity (both in space and time).

Organic plant breeding and seed production

As explained earlier, the production systems differ significantly between organic and conventional farms. These differences create a need for plant cultivars adapted to their specific production environment. The use of cultivars that are bred to produce maximum yields in a conventional system with high external inputs of mineral fertilizers and chemical plant protectors might not always be suited for organic conditions where these types of external inputs cannot be used. The difference in the farming systems creates a demand for cultivars adapted for low-input organic conditions but also the idea of keeping the whole production chain organically produced (not only the crop itself but also the seeds). Unless the farmer produces his or her own seeds, there are four different types of seed producers that generally can be distinguished (Lammerts van Bueren E.T. and Myers R. 2012):

- Completely independent organic seed companies.
- Semi-independent daughter companies connected to a conventional seed company.
- Conventional seed companies with an integrated organic part.
- Conventional seed companies without organic seeds.

When it comes to the breeding of new varieties - the cultivars that end up being marketed as organic cultivars can be created out of three different scenarios (Wolfe M.S. et al., 2008):

- Conventional breeding programs but the cultivar turns out to be suitable for organic farming conditions as well.
- Conventional breeding programs that are aiming to produce varieties suited for organic and low input conditions.
- Organic breeding programs that are conducted under organic conditions.

Since organic agriculture operates under a different set of ethics and values compared to conventional agriculture the breeding techniques and tools that can be used also differs. The following list describes recommended principles for organic plant breeding and comes from Wilbois P.K. *et al.* (2012) and is based on two reports by Lammerts van Bueren E.T. *et al.* (1999) and Wyss E. *et al.* (2001):

- Crossing and selection techniques at the whole plant and crop level are most appropriate for organic plant breeding.
- Crossing techniques are appropriate in an organic plant breeding system provided that pollination and seed formation occur on the plant growing in soil.
- F_1 hybrids may have a role in organic farming provided that the F_1 is fertile and that the parent lines can be maintained under organic growing conditions.
- Techniques at the cell level (in vitro techniques) are generally considered inappropriate for organic breeding as they operate beyond the context of organic production systems.

- Genetic engineering is not allowed in organic sector. It operates below cellular level and no longer in the direct context of life and therefore violates the integrity of plants.
- DNA diagnostic methods such as molecular marker-assisted selection can in principle supplement other selection methods used in organic plant breeding such as selection based on morphological traits, or disease screening tests with inoculation of the pathogen, or by protein detection in baking quality tests.

The differences between conventional and organic production systems also lead to differences in the specific breeding goals in terms of plant ideotypes. The following differences between the two cultivation methods in this section are presented in Lammerts van Bueren E.T. and Myers R. (2012). While conventional breeding programs aims to find cultivars that performs well at high population densities with high inputs of synthetic plant protectors and mineral fertilizers it is important for organic systems to have cultivars optimized for lower plant densities with nutrients from organic sources. Both systems aim to increase resistance and tolerance towards pests and disease although the different management methods might give importance to different types of resistance or threats. Both conventional and organic systems want to increase harvest index although organic systems might also acknowledge and value the incorporation of crop residues to retain nutrients and build soil organic matter. Conventional systems often benefits from erect plant and leaf architecture with short plant stature while organic systems often benefit from taller plants and a more spreading canopy to compete with weeds. While the roots often are of less importance when breeding conventional cultivars they play a more important role for organic cultivation. Conventional cultivars need root systems that can obtain as much nutrients from readily available nutrients (mineral fertilizers) as possible while organic cultivars need to be able to obtain nutrients from mineralization (that is not readily available) as well as plants with high nutrient use efficiency. Since legumes provide an organic source of nitrogen this aspect could also be good to include when breeding legumes. Plant genotypes associated with inefficient rhizobia symbiosis should be discarded from further developments. Since working conditions might differ between conventional and organic production systems aspects such as harvest and weeding methods could also be included when designing plant architecture.

Pests & Diseases

Pest and disease resistance (and tolerance) are very important aspects to plant breeding and even more important to plant breeding aimed for the organic sector (Lammerts van Bueren E.T. and Myers R. 2012). If an organic breeding program for cowpea will be started in Portugal it would be essential to investigate which threats there are to cowpea production in the region as well as a risk assessment of new threats that could come in the future. This type of work as well as other aspects of pests and disease management and breeding has not been included in this thesis mainly because it is such a big topic in itself and the practical trials would require preparations and tools not available at the time that this research were performed.

Breeding methods

This section briefly presents some breeding methods that could be suitable for a small scale organic breeding program of cowpea. It usually takes to about the F_6 generation of inbreeding and some generations of yield testing before the breeding line can be released as a new cultivar (Hall A.E. *et al.,* 1997). Depending on the breeding objective different methods might be more suitable than others and they can also be combined and customized to fulfil their purpose. Hall A.E. *et al.* (1997) propose the following breeding methods together with the explanations presented in this section (as well as some other breeding methods for cowpea that are not suited for organic breeding and have therefore been excluded in this paper) (any facts stated in this section comes from this source unless another one is provided). *Line selection within landraces* aims to select superior lines from land races or old cultivars that could be genetically variable. The selected lines could then be used to make a variety in itself or as a parent for crossing. This method is beneficial due to its already fixed genetic composition which more or less makes it possible to leave out the initial F_6 generations of selfing (self-fertilization) and start directly with yield trials. On the other hand the genetic variation within the landraces and old cultivars might be limited and it can be difficult to find new useful traits there. The most common approach to breed cowpea is *pedigree breeding* which is based on an initial cross between two parents followed by many generations of selfing and single plant selection. Exotic parents are often used in the early stages of breeding programs to introduce new genetic material that are not already present within the locally adapted cultivars. In case only one or a few traits are desired from one of the parents *backcross breeding* can be applied by crossing the progeny of each generation back with the parent of which the main plant character should be remained. For every generation of backcrossing half of the donor germplasm is reduced. After four generations of backcrossing the new progeny would have about 97 % identical germplasm as the parental line it was backcrossed to. With good selection work the remaining 3 % should include the new desired genes. These methods are used to create specific cultivars but it is also possible to grow cowpea in populations with diverse genetic composition (see figure 2). The *bulk method* starts with crosses being made and then harvests all the seeds from all the plants generation by generation. Natural selection will favour competitive traits and with each generation the population will become more homozygous. The bulk method is supposedly best when the nursery conditions impose strong selection pressure for important traits (this aspect will be further discussed in the section 'Breeding cowpea for intercropping'). Another important aspect is that bulk populations maintain genetic diversity and therefore contributes to a higher degree of resilience towards fluctuations in environmental factors (Dawson J.C. and Goldringer I., 2012). It is also worth adding *participatory plant breeding* to the list of potentially useful breeding methods. It might be most useful combined with the bulk method but it could be interesting to investigate the opportunities of combining it with any of the methods that will be chosen for the breeding program. In general participatory plant breeding could be generalized as involving farmers from an earlier stage for setting objectives and taking part in selections as well as decentralizing the breeding to different locations in order to favour adaption to local environments (Desclaux D. *et. al.,* 2012). Hall A.E. *et al.* (1997) states that agronomic evaluations indicate that cowpea cultivars developed for a certain region of the world might not be suited for other regions. This statement reinforces the idea that decentralized (participatory) methods might be beneficial to a cowpea breeding program.

Potential germplasm sources

There are several places that can contribute with useful cowpea germplasm for a breeding program. INIAV (the Portuguese agricultural gene bank) have cultivars that they are willing to share without restrictions but also cultivars that require a legal contract before they can be shared (personal communication: Ana Maria Barata, 2016). There is also the option to get cultivars from farmers and private collections. There is a private seed conservatory organisation that has a lot of old Portuguese varieties but is not open to share them with commercial companies. Having access to traditional varieties adapted to the Portuguese environment could be very useful when developing new cultivars adapted to climate change and organic agriculture (also taking in to consideration that these traditional varieties have been cultivated organically by default in the past). Using these traditional cultivars in breeding programs could allow the development of new pest and disease resistant, as well as heat tolerant, varieties with traditional Portuguese seed types (as well as other valuable traits). There is also the alternative of getting cultivars from other seed companies and use them for breeding. This option has the advantage of also getting information about plant architecture and potential pest and disease resistance. Similar to the option of getting cultivars from seed companies'; universities, research institutes, and gene banks from other countries also can contribute with germplasm material with known and useful properties (Filho F.R.F. *et al.,* 2012; Hall A.E., 2011). Some very significant germplasm collections of cowpea are the USDA (United States Department of Agriculture) which had about 7 000 accessions and UCR (University of California, Riverside) which had about 5 500 accessions in 2001 (Hall A.E.*et al.,* 2003). IITA (International Institute of Tropical Agriculture), which might have the biggest germplasm collection of cowpea in the world, has collected and conserved over 15 000 accessions from over 100 countries including over 500 accessions of related wild species (Singh B.B. 2007).

Breeding goal suggestions

Since the Sementes Vivas cowpea project was starting during the start-up year of the company itself (which required a lot of work and attention from all the employees) it became a bit of a side story with an uncertain long term objective. It was (and is) not clear whether the goal is to simply find already existing cultivars suited for different cultivation methods (sole cropping and intercropping) as well as different market segments (small scale farmers, large scale farmers, and home garden) or to start up an actual breeding program to create cultivars suited for future demands of organic and biodynamic agriculture. Which decision that will be made based on the financial opportunities and restrictions associated to the project is outside the scope of this thesis. This section suggests trial and breeding objectives potentially suited for Sementes Vivas small scale breeding (and associated to organic agriculture demands) program that could be further investigated in regard to their financial feasibilities:

- Find already existing or develop cultivars suited for intercropping and sole cropping (organic and biodynamic production methods) in Portugal and the surrounding region.
- Incorporate resistance genes to existing and potential future pest and disease problems.
- Increase yield and potentially biomass (for hay varieties and green manure).
- Breed fast developing varieties that could potentially be used in alternative crop rotation schedules (for food, animal feed, or green manure).
- Develop heat tolerant varieties adapted to a climate change scenario of rising temperatures.
- Find or breed cultivars suited for mechanical harvesting.
- Find or breed cultivars that are competitive against weeds.
- Find or breed cultivars with short cooking time (which are more sustainable since they consume less energy to be prepared).

Crossing method

Although no crossings were conducted during this trial a method is included in this report to facilitate the work for future breeders working on the project. The artificial pollination of cowpea is supposed to be relatively easy. Ehlers J.D. and Hall A.E. (1997) present the following way that has had more than 90 % pod set when conducted in a greenhouse. In the afternoon of the day before the artificial pollination is supposed to take place the flowers that are expected to open the next day is selected and emasculated. To emasculate the flowers the sepals and keel is opened and the anthers are removed. All of this is done with tweezers and it is important not to damage any of the anthers during removal since this can lead to self-pollination. The pollination preferably takes place the next morning between 7 - 10 am which is the time of highest success rate. The emasculated flowers that have been left open during the night are pollinated by taking open flowers by the desired male parent and brushing its pollen on to them. Male parents can also be chosen during the morning of the same day as pollination and refrigerated at 4 to 10 ^{o}C until the evening when they are used to pollinate the emasculated flowers. Night temperatures above 20 ^{o}C reduce the success rate of the pollination (Warrag M.O.A. and Hall A.E., 1983).

Breeding cowpea for climate change adaptation

One of the biggest challenges we are facing today as the human race but also for numerous other living species might be the known and unknown effects of climate change. There are plenty of indications of changes that could have an impact on the performance of cowpea production all over the world. Plant breeding is a process that takes a long time (often decades) and therefore potential changes in the climate would be appropriate to take into consideration when designing new cultivars. There are both potentially positive and negative aspects of the climate change that can influence future cowpea production. If temperature raises new areas of land might become potential production sites while others might become too hot for our current cultivars. Selecting or breeding cultivars adapted to the growing conditions of these new sites (that used to be too cold before) should generally not be too hard since there is a wide range of cowpea cultivars and genotypes that could be relatively easy to test in local trials. Breeding cultivars that can manage to grow in the areas that might get too hot for the currently existing cultivars may be more of a challenge but yet a very important task. Most of the world’s cowpea production takes place in tropical areas of sub-Saharan Africa but significant production also takes place in tropical areas of Brazil and Asia as well as subtropical areas of United States and South Africa (Singh B.B. et al., 2002). Since the growing conditions in some of these areas are very harsh already, changes in the climate could have significant effect on future yields. Portugal as well as the other cowpea producing countries in Southern Europe can also get affected by changes in the climate. The temperature of this area could exceed a 6 ^{o}C increase during summers by the end of this century (Alcamo J. et al. 2007). The increased temperature together with projected decreases in summer precipitation can also lead to heat waves and droughts (Alcamo J. et al. 2007).

The vegetative stages of the cowpea life cycle are generally very heat tolerant while the reproductive stages are more sensitive to high temperatures (Hall A.E., 2004). There are several studies concluding that it is the high night temperatures that are stopping or reducing the pod setting abilities (Hall A.E., 2011). In a trial by Nielsen C.L. and Hall A.E. (1985) they found that there was a linear decrease in grain yield from 2.8 to 1.4 t ha^{-1} and a linear decrease in pod set from 62 % to 32 % when the minimum night time temperature went from 15 ^{o}C to 26.5 ^{o}C. This equals a 4.4 % decrease in grain yield per degree Celsius increase in night time temperature starting from 15 ^{o}C. There are also studies showing a reduction in number of seeds per pod under hot night conditions (Hell A.E., 2011) and for some genotypes the floral bud development stops under long day and very hot field conditions (Patel P.N. and Hall A.E., 1990). Some genotypes initiates flowering due to photoperiod and does not flower during long day conditions. These genotypes have the capacity to produce flowers even if the night temperate is high (if the right photoperiodic conditions are provided) (Mutters R.G. et al., 1989).

Due to the potential temperature raises mentioned earlier there might be a need to breed cultivars that have reproductive stages tolerant to warmer climates. There are heat tolerant cultivars available to be used in breeding programs (Hall A.E., 2011). These cultivars could be used to combine the heat tolerance trait with other desired traits.

There are also potentially positive (in terms of agricultural yields) effects from the projected climate changes. C3 plants such as cowpea exhibits increases in their photosynthetic systems with increased levels of CO_2 in the air. This could lead to higher productivity of the plants since raising CO_2 levels is a projected feature of the changing climate (Hall A.E., 2011).

Developing cowpea intercropping systems for climate change mitigation & environmental sustainability

The reason why intercropping systems with cowpea (and legumes in general) can be motivated in a climate change mitigation perspective is due to the relatively low greenhouse gas emissions and energy usage these production systems (legume based N-fixation) create in comparison to production systems where the N-fertilizers are created in high energy consuming processes driven by fossil fuels (Jensen E.S. *et. al.*, 2011). Tariah N.M. & Wahua T.A.T. (1985) has shown that the land equivalent ratio (LER) (see section 'Land equivalent ratio (LER)' below for further explanation) of maize barely gets affected by intercropping with cowpea. This gives us an opportunity to grow a plant that fixates N from the atmosphere simultaneously as we grow the maize. This extra crop could then be used as food, animal fodder, or directly be put back into the ground as a green manure. By optimizing these systems we optimize a way to fertilize the soils and increase productivity without using nitrogen that comes from high energy consuming processes driven by fossil fuels.

The optimization of the intercropping systems with cowpea could be an essential part in offering an alternative to high input farming. There are already plenty of reports that indicate higher productivity when cereals are intercropped with cowpea (Takim F.O., 2012; Tariah N.M. and Wahua T.A.T., 1985). These studies often investigate one aspect at a time. By creating a program with the objective to develop the intercropping practise by optimizing multiple factors a more competitive sustainable farming practise could potentially be obtained. Aspects that could be useful to investigate and optimize include:

- Relative sowing time between the companion crops.
- General sowing time for the companion crop combination.
- Cultivars selection of both the companion crops.
- Breeding new cowpea cultivars (or both the crops).
- Cultivar selection of both companion crops in relation to site specific environmental conditions.
- Sowing and harvest methods.

By optimizing the intercropping practise the farmer willingness to adopt the new method would hopefully increase. There are many reasons why it is important to incorporate cowpea/cereal intercropping as a modern farming practise (especially for organic farmers). The use of multiple crops simultaneously increases the domestic biodiversity but also promotes wild biodiversity; the cowpea is working as a cover crop between the cereals which functions to supress the growth of weeds and hence has the potential to reduce the usage of herbicides (for conventional agriculture) that otherwise can leech out and damage the wildlife biodiversity in the surrounding environment (Marshall E.J.P., 2001). In organic agriculture the weed suppression ability could rather reduce the need of mechanical weeding. Since intercropping with cowpea increase both seed and biomass production of the cultivated area it could help to increase the production of not just human food but also animal fodder or green manure that can be incorporated back into the soil to increase the level of organic matter (John P.S. *et. al.,* 1992). Higher level of organic matter is commonly known to prevent soil degradation and soil erosion. A study conducted with cowpea and sorghum showed benefits related to water runoff and soil loss when the two crops were used together in an intercropping system (Zougmore R. *et al.,* 2000). As mentioned earlier the cowpea also fixates atmospheric nitrogen that helps fertilizing the land and balancing the C:N (carbon to nitrogen) ratio of the soil. It has also been shown that intercropping cereals with cowpea increases the phosphor (P) availability in alkaline soils (Latati M. *et. al.,* 2014).

Land equivalent ratio (LER)

Land equivalent ratio (LER) is the value that is commonly used when comparing different intercropping scenarios and indicates the efficiency of land use when growing two (or more) different crops or varieties together compared to growing each crop separately on the same land area. LER can be explained with the formula (Mead R. and Willey R.W., 1980):

$$LER = L_A + L_B = \frac{Y_A}{S_A} + \frac{Y_B}{S_B}$$

Where L_A and L_B are the LERs of each individual crop, Y_A and Y_B are the individual crop yields in intercropping, and S_A and S_B are the individual yields in sole cropping. A LER value of 1.3 would for instance indicate a yield increase of 30 % but it does not indicate how much each crop contributes to the increase. It does not indicate other aspects such as economic or nutritional values.

Intercropping cowpea & maize

There are numerous reports showing promising effects of maize and cowpea intercropping systems. Saudy H.S. (2015) concludes beneficial effects such as enhanced weed suppression, boosting of maize grain yield as well as maximizing nitrogen and cultivated land usage. When maize and cowpea are grown together in an intercropping system the maize will be the dominant crop. It has been shown that the LER (land equivalent ratio) of the maize only reduce slightly in intercropping systems with cowpea while the LER of the cowpea will have a more significant reduction (Waitiki J.M. *et. al.*, 1993). The same study also concluded that the use of different cowpea cultivars had no effect on the maize yield but played an important role on the cowpea yield; this suggests that high yielding cowpea cultivars can be used without any reduction of the maize yield. The population density of cowpea does not have a significant influence on the maize yield when the two crops are grown together (Tariah N.M. & Wahua T.A.T., 1985). Optimum population densities are shown to be approximately 20 000 maize plants ha^{-1} and 33 000 cowpea plants ha^{-1} with a LER of about 1.48, but the whole range of population densities tried in that study (18 520 – 55 560 plants ha^{-1} for maize and 20 000 – 66 660 plants ha^{-1} for cowpea) gave a LER value between 1.40 and 1.50 (Tariah N.M. & Wahua T.A.T., 1985). The small difference in LER for different plant populations indicates a possibility to adapt the population densities to other growth factors (e.g. water availability and soil quality). When designing the intercropping system it is important to take the maize genotype and relative sowing time into consideration so that optimum light interception is allowed to the cowpea canopy during its reproductive stages (Ewansiha S.U. *et al.,* 2014).

Intercropping cowpea & other crops

Cowpea has a tradition of being intercropped with different options of companion crops. Some of them have the same or even higher potential as companion crops as maize but are still mentioned here in a shorter manner since the main focus of Sementes Vivas (see section 'Sementes Vivas') has been cowpea together with maize during this stage of the project.

Cowpea and millet intercropping is for instance the most predominant cropping system in the Sudan and Sahelian zones of West Africa (Henriet J. *et al.,* 1997). When millet and cowpeas are grown together the scenario is similar to the combination of maize and cowpea in the sense that the millet will be dominant over the cowpea. There also seem to be little or no impact on millet productivity in relation to different cowpea genotypes (Mohammed I.B. *et al.,* 2008; Craufurd P.Q., 2000) which indicates good opportunities to freely select the best performing cowpea cultivar without disturbance on the millet yield. Sorghum is also a crop well known to be intercropped with cowpea with plenty of research conducted on the combination. Some examples of positive benefits shown from research on this combination are reduced water runoff and reduced soil loss compared to both crops respective sole cropping systems (Zougmore R. *et al.,* 2000). Even if maize, millet, and sorghum seem to be the most significant companion crops for cowpea other crops such as okra and cotton has also been seen in research articles. Muoneke C.O. *et al.* (1997) showed positive results for the intercrop yields when comparing LER between okra and cowpea intercrop systems with their respective sole cropping systems.

Breeding cowpea for intercropping in Portugal

In order to give intercropping systems a fair comparison to other highly researched and developed cropping systems one could argue that it is essential to first select or develop cultivars that are properly adapted to intercropping. Cowpea has traditionally been used in intercropping systems in Portugal although this practise is not common today (personal communication: Jose Amorim, 2016). If this is correct it might be an indication that some of the traditional Portuguese cultivars already could have a certain level of adaptation towards intercropping systems. Organic cowpea gives a higher income per hectare than maize (which in this case would be the primary crop to develop an intercropping system with) in Portugal (personal communication: Manuel Sousa Fernandes, 2016). This indicates that cowpea most likely only would be intercropped in a scenario where it can be included as a bonus crop when maize has to be grown since it otherwise would me more profitable to simply grow the cowpea as a sole crop. The initial step to create an intercropping breeding program should therefore include defining which types of maize (or any other potential companion species) that will be used and how they are cultivated. When knowing that, it will be easier to create a defined ideotype for the new cowpea cultivar. Hall A.E. *et al.* (1997) mentions examples such as photosensitive cultivars that start flowering just after the cereals begin to senesce or extra early cultivars that matures before the canopy of the cereals has fully developed. They also underline the importance of the interaction between the cowpea genotype and the genotype of the companion crop. Another example is a trial conducted by Ewansiha S.U. *et al.* (2014) in Nigeria which concluded that the intercropped cowpea yields were lower when intercropped with late maze that maintained a high leaf are over a longer period of time. These sources indicates that the cultivar of the companion crop should be carefully chosen to facilitate high cowpea yields by finding ways to allow as much light interception as possible during the reproductive stages of the cowpea.

It is more difficult to breed for intercropping since there are more environmental factors compared to sole crop breeding (Hall A.E. *et al.,* 1997). Since the target environment is more complex it might be difficult to consciously predict which specific traits to incorporate into one cultivar. Nelson C.S. and Robichaux R.H. (1997) mention that selection for high yields in sole cropping systems might not lead to high yields in intercropping systems. They also conclude that spreading growth habit, number of branches, number of nodes, and internode length are associated with higher yields in intercropping systems. They explain that the ability to form pods on branches and nodes further away from the main stem might be beneficial to form pods where there is a higher degree of light interception and that many cultivars developed for sole cropping are designed to produce pods close to the main stem to prevent lodging.

Another option that could be interesting to investigate as a breeding method for intercropping is the bulk method (previously explained in the section 'Breeding Methods'). No references in literature have been found of this method being used in order to breed for intercropping systems but some statements from Hall A.E. *et al.* (1997) indicates that it might be suitable. They say that the bulk method is best suited when the nursery conditions impose strong selection pressure for important traits which might be assumed for the intercropping nursery. They also say that spreading growth habit normally competes better in variety mixtures which normally is not desired for sole cropping systems but it is the common growth habit for cowpea in intercropping systems. Perhaps the bulk method could be a cost efficient way to find cultivars or variety mixtures well suited for intercropping by letting natural selection bring (perhaps together with some strategic selections) out the best adapted genotypes.

Traits

The main goal of plant breeding is to design cultivars adapted to a certain objective. This is basically done by introducing or keeping genes that map certain desired qualities and removing genes that map undesired qualities in the progeny. Each trait represents a quality in the plant that is controlled by one or multiple genes. Depending on the breeding objective and the target cultivation methods different traits have different significance. There is for instance a general consensus for most agricultural categories would be that the yield level is very important (although higher quality levels may compensate for lower yields with higher prices). There has been research conducted on cowpea correlating certain traits with yield levels. Some of the traits concluded to be directly correlated to yield are pod length, number of pods per plant, seeds per pod, and pod weight (Romanus K.G. *et al.,* 2008; Oladejo A.S. *et al.,* 2011). Knowing how different traits correlate to certain aspects of the plant (such as yield or disease resistance, etc.) is important in order to make strategic crosses to develop better cultivars.

Thesis objective

The objective for the thesis is to characterise and evaluate the genetic material from the cowpea germplasm collection of Sementes Vivas under sole crop organic farming conditions in Portugal as well as investigating further potential usage and development of this material in relation to organic farming, sustainable development, and plant breeding.

Hypothesis

The germplasm collection of Sementes Vivas contains genetic variety that can be useful for improving and developing different types of organic cowpea cropping systems in Portugal.

Research questions

Questions that should be answered from the trials and evaluations are:

- Which accessions has valuable traits that can be taken into consideration for breeding and development; traits associated to organic agriculture, sole cropping and intercropping.
- Investigate general aspects of the germplasm collection; characterising the accessions by looking at qualitative and quantitative traits.

Material & Method

Trial design & location

The trials are conducted on the Sementes Vivas farm (see figure 5) located at Couto da Várzea, Idanha-a-Nova in Portugal. The trial field had silty loam soil with a pH of 5.8 and 2 % organic matter. No inoculation of rhizobia or fertilisers was used. The seeds were sown August 5th 2016 (and cancelled November 1st 2016) in beds of 45 m x 1.2 m with 0.2 m marginal to the sides, 0.3 m between the sown seeds, and 0.8 m distance between then rows. Each accession had 2.25 m long plots (14 sown seeds) except accession 15 that were filling out the end of the first row. The smaller earlier trial conducted by an employee at Sementes Vivas were sown June 14th 2016; this trial were not included in this thesis except for a few qualitative results (if a result comes from this trial it will be mentioned in the result table). The trial was set up with one plot for each accession (see figure 3). The trial was irrigated once a day from sowing until the 13th of September when the irrigation tubes was removed and then the trial was non-irrigated for the rest of the time. Removal of light competing weeds was conducted by hand while lower ground covering plants were left to grow. Figure 6 indicates the temperature development and figure 7 indicates the rainfall for Idanha-a-Nova during the trial (this data comes from a website, and is provided as an indication of the weather development and cannot be guaranteed to be totally accurate values) (World Weather Online, 2017). Comments on the trial design can be found in the discussion section under 'Trial design discussion'.

39	37	36	35	34	33	32	30	29	28	27	26	25	24	23	22	21	20	19	18
6	16	17	38	1	2	3	4	5	7	8	9	10	11	12	13	14	15		

Figure 3. Spatial arrangement of the accessions in the field plots.

Figure 4. Trial location in Portugal (source: Google, 2017).

Figure 5. Sementes Vivas farm and the cowpea trial field (marked with red) (photo: Micha Groenewegen).

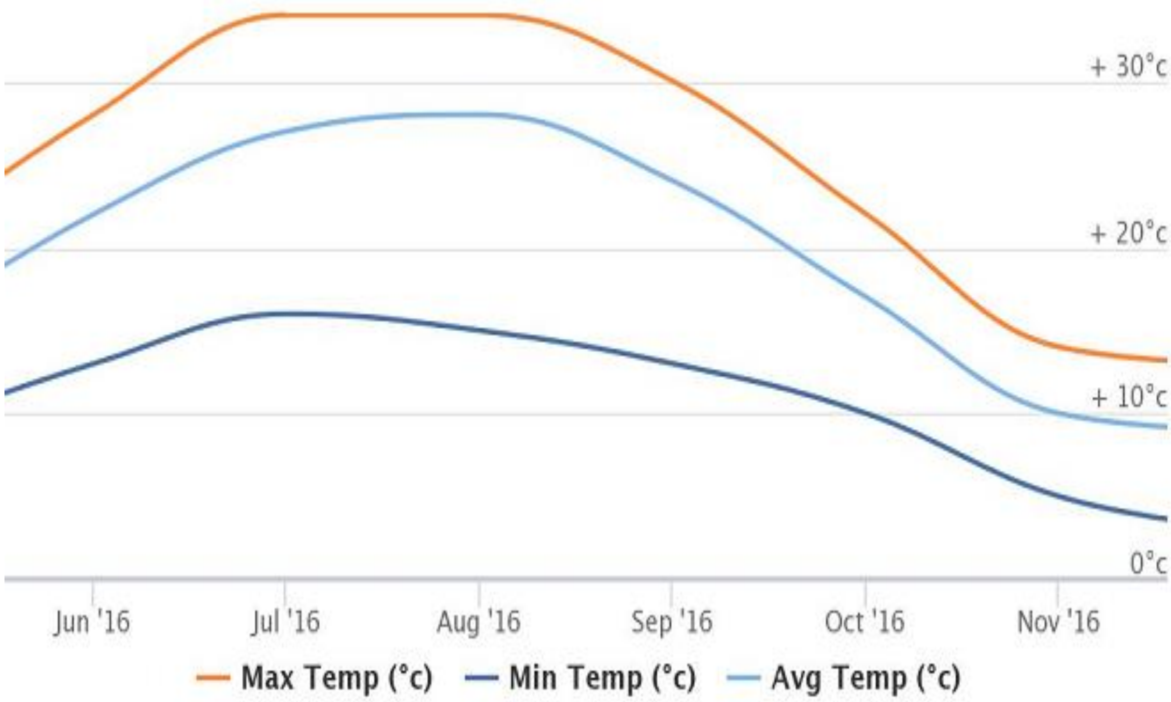

Figure 6. Temperature in Idanha-a-Nova during the trial (source: World Weather Online, 2017).

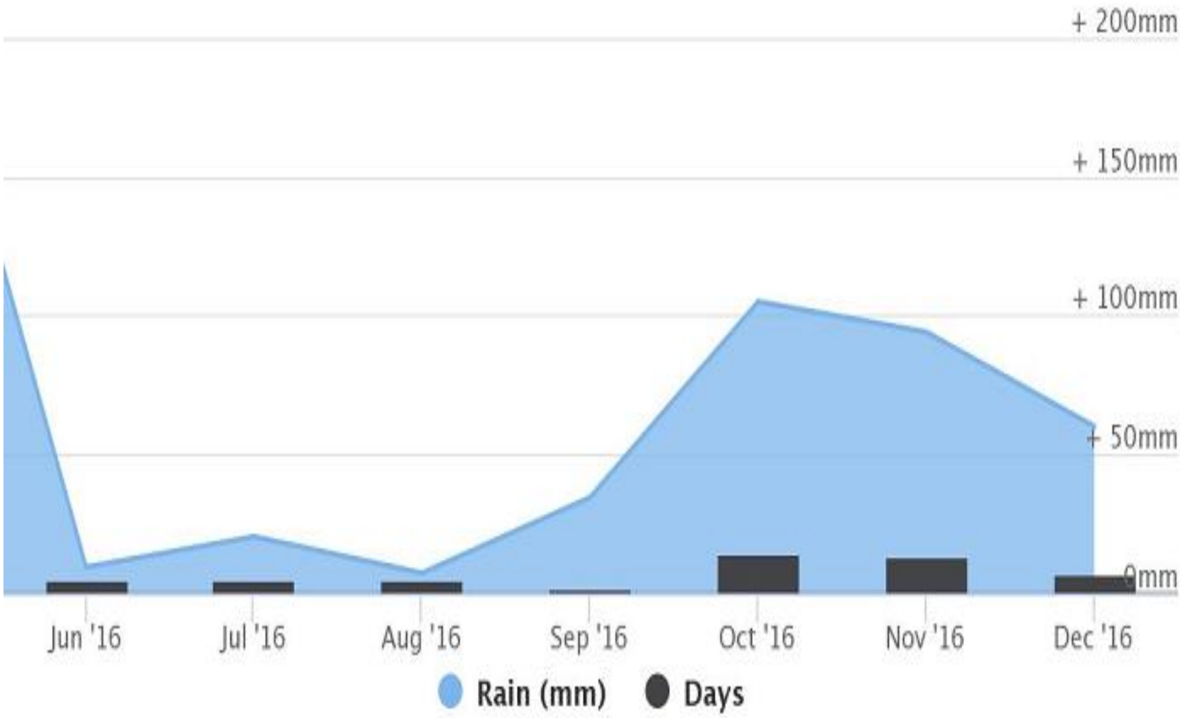

Figure 7. Rainfall in Idanha-a-Nova during the trial (source: World Weather Online, 2017)

Germplasm sources

The seeds of the different accessions used in this trial come from different sources mainly in Portugal but also from other places of the world. In some cases the seeds has come with no or limited information (growth habit, yield, variety name, etc.) and in some cases there has been a variety description available (when ordered from seed companies).

Table 1. Germplasm sources

Acc.	Origin	Variety Name	Remark
1	Unknown	Unknown	
2	Unknown	Unknown	
3	Unknown	Unknown	
4	Unknown	Unknown	
5	Linong Seeds (China)	No. 312	ssp. *sesquipedalis* (bush type)
6	Linong Seeds (China)	No. 324	ssp. *sesquipedalis*
7	Organic shop (Portugal)	Caraverde	Variety name not certain
8	Senhora do Almortao market (Portugal)	Unknown	
9	Organic shop (Portugal)	Bago de Arroz	
10	Senhora do Almortao market (Portugal)	Unknown	
11	Portuguese supermarket (Unknown)	Unknown	
12	Herdade D Carvalhoso Alentejo (Portugal)	Unknown	
13	Flora Lusitana (Potugal)	Unknown	
14	Portuguese supermarket (Peru)	Unknown	
15	Ingegnoli (Italy)	"Fagiolo Dell'occhio"	
16	Vreeken's Zaden (Netherlands)	Purple Mart AB-110	ssp. *sesquipedalis*
17	Vreeken's Zaden (Netherlands)	Metro Black	ssp. *sesquipedalis*
18	Vreeken's Zaden (Netherlands)	KY Bush	ssp. *sesquipedalis* (bush type)
19	Vreeken's Zaden (Netherlands)	Top Pick Pinkeye	
20	Vreeken's Zaden (Netherlands)	Mississippi Silver	
21	Vreeken's Zaden (Netherlands)	Mississippi Purple Brown Crowder	
22	INIAV (Portugal)	ACC No. 03522	
23	INIAV (Portugal)	ACC No. 03521	
24	INIAV (Portugal)	ACC No. 03441	
25	INIAV (Portugal)	ACC No. 03470	
26	Local shop in Castelo Branco (Portugal)	Bago de Arroz	
27	Local shop in Castelo Branco (Portugal)	Unknown	
28	Local shop in Castelo Branco (Portugal)	Unknown	Separated from acc. 27
29	Organic farmer in Portallegre (Portugal)	Unknown	
30	Organic farmer in Portallegre (Portugal)	Unknown	
31	Ramgo Seeds (Phillipines)	Pole Sitao Conception	ssp. *sesquipedalis*, not included in main trial
32	Farmers market in Sao Pedro du Sol (Portugal)	Unknown	Acc. 32-37 are from same bag
33	Farmers market in Sao Pedro du Sol (Portugal)	Unknown	Acc. 32-37 are from same bag
34	Farmers market in Sao Pedro du Sol (Portugal)	Unknown	Acc. 32-37 are from same bag
35	Farmers market in Sao Pedro du Sol (Portugal)	Unknown	Acc. 32-37 are from same bag
36	Farmers market in Sao Pedro du Sol (Portugal)	Unknown	Acc. 32-37 are from same bag
37	Farmers market in Sao Pedro du Sol (Portugal)	Unknown	Acc. 32-37 are from same bag
38	Unknown (Netherlands)	"Korte Kouseband"	ssp. *sesquipedalis*
39	Unknown	Unknown	

Measurement methods

Growth habit

Growth habits were evaluated two times with two different ways to describe them. Explanation to this can be found in the discussion section under 'Growth habit and twinning tendency'.

1st evaluation:

Recorded on the 6th week after sowing. Categorization based on the IBPGR Descriptor (IBPGR Secretariat, 1983) for cowpea:

1. Acute erect (branches form acute angles with the stem)
2. Erect (branching angles less acute than above)
3. Semi-erect (branches perpendicular to main stem, but not touch the ground)
4. Intermediate (most low branches touch the ground)
5. Semi-prostrate (main stem reaches 20 cm or more above ground)
6. Prostrate (plants flat on ground, branches spread several meters)
7. Climbing

2nd evaluation:

Evaluated by the end of the growing season and distinguished in three categories (bush, spreading, and climbing) by making eye observation of the overall appearance.

Days to flowering

Date recorded of the day of first flowering and on the day when 50 % of the plants has begun to flower.

Flower colour

Observed on fully developed flowers. Based on personal judgement of the predominant colour.

Testa (seed coat) texture

Observed on seeds before sowing and categorized by:

- Smooth
- Smooth to rough
- Rough (fine reticulation)
- Rough to wrinkled
- Wrinkled (coarse folds on the testa)

Eye pattern

Observed on seeds before sowing and classified by thin, medium or large around hilum or described in other words if necessary.

Eye colour

Observed on seeds before sowing and described based on personal classification (no colour index or similar tool were used).

100-seed weight

Weight of 100 representative seeds (collected from different sources and not produced under similar treatment) measured before sowing.

Plant vigour

Recorded 3-4th week after sowing on a scale of 1 to 3 (low, medium, high) based on eye observations and scaled in relation to each other.

Number of main branches

Measured on the 25th October. Number of branches located on the main stem that has reached a length of 5 cm or more.

Length of main stem

Measured on the 25th October. Length measured from the first node to the tip of the main stem (see figure 8).

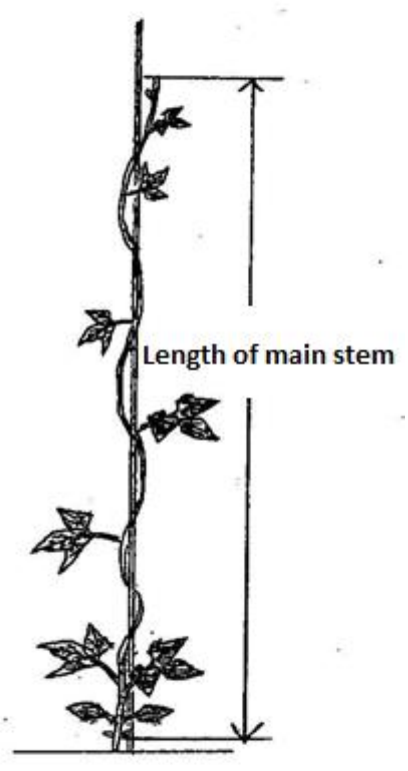

Figure 8. Length of main stem measurement points (source: UPOV, 2009).

Immature pod pigmentation

Observed on the fresh pods (some results were obtained from a trial sown earlier during the same year; these have been marked in the result table).

Seed length

Mean value of five representative seeds, measured before sowing. See Figure 9.

Seed width

Mean value of five representative seeds, measured before sowing. See figure 9.

Seed thickness

Mean value of five representative seeds, measured before sowing.

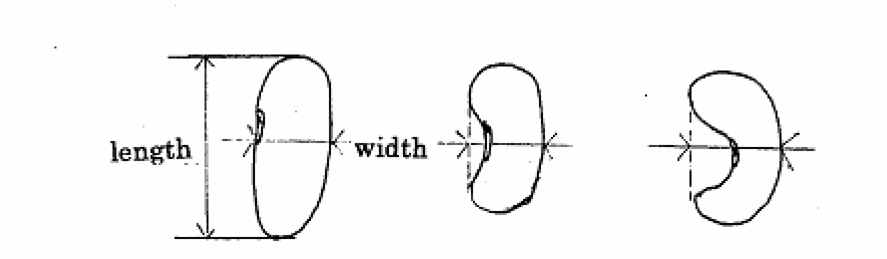

Figure 9. Seed length and width measurement points (source: UPOV, 2009).

Seed shape

Observed on collected seeds before sowing. Categorization based on the IBPGR Descriptor (IBPGR Secretariat, 1983) for cowpea:

1. Kidney
2. Ovoid
3. Crowder
4. Globose
5. Rhomboid

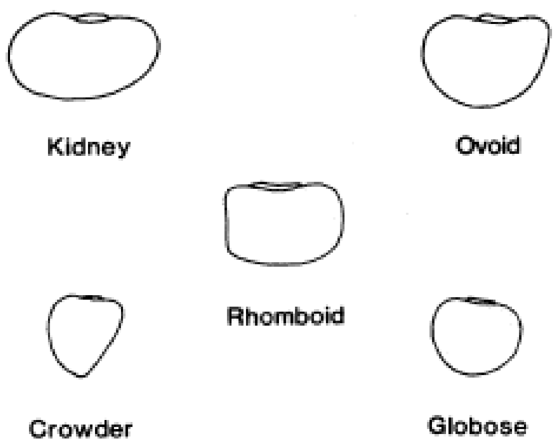

Figure 10. Cowpea seed types (source: IBPGR Secretariat, 1983).

Petiole & Petiolule length

Mean value from the 5th node on five representative mature plants (leaf fully developed). The petiole is measured between the base of the terminal leaflet and the base of the two side leaflets (see figure 11). The petiolule is the stem that carries the leaf from the node on the plant to the base of the two side leaflets (see figure 11).

Terminal leaf length and width

Mean value from the terminal leaf on the 5th node from five representative mature plants (leaf fully developed). The terminal leaflet length is measured between the top and the base of the terminal leaflet (see figure 11). The terminal leaflet width is measured with the ruler over the widest area of the leaf in a 90° angle to the middle vein (see figure 11).

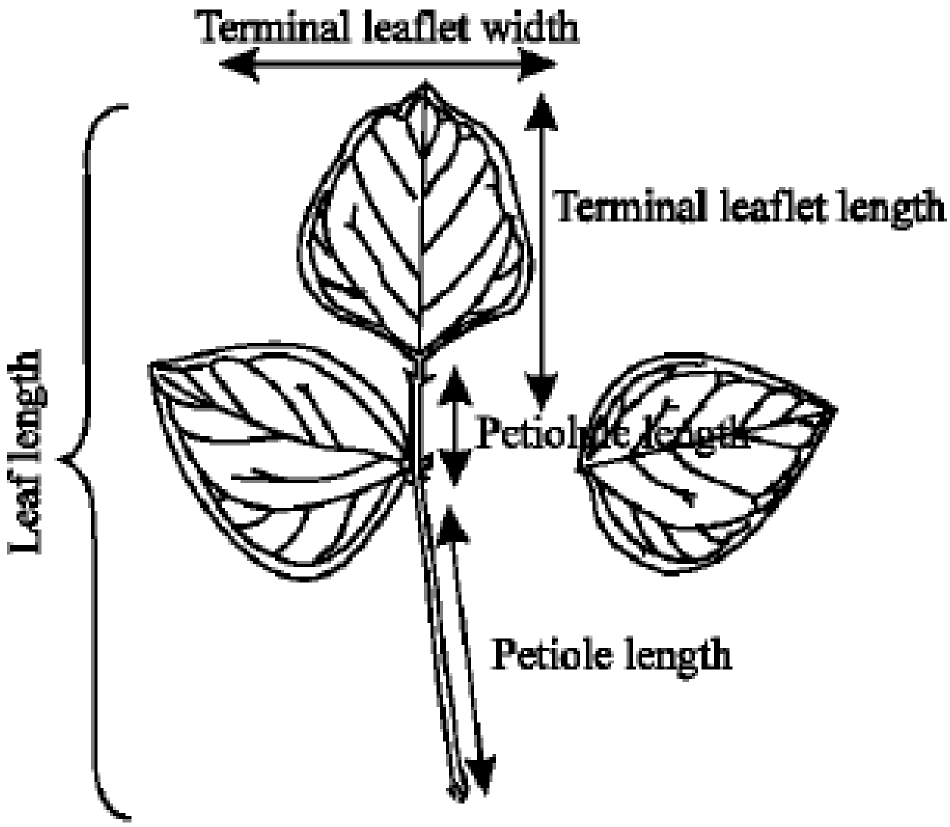

Figure 11. Cowpea leaf parts (source: Pekşen E. *et al.,* 2005).

Leaf colour

Observed on the 13th October. Categorization based on the IBPGR Descriptor (IBPGR Secretariat, 1983) for cowpea:

- Pale green
- Intermediate green
- Dark green

Statistical analysis

The collected data were analysed with Excel and Minitab 17. Fisher’s LSD test was used to group the results based on significant difference for length of main stem, number of main branches, petiole length, petiolule length, terminal leaflet length, and terminal leaflet width. The measured values are based on pseud-replications obtained from five representative (excluding damaged or exceptionally weak) plants within the same plot. The seeds are obtained from diverse sources and the data is expressed in mean values obtained from five representative seeds (except for 100-seed weight). No statistical analysis have been conducted on the seed data; they are only included as indication values.

Results

Seed results

Figure 12 shows the seed collection of Sementes Vivas. Accession 39 was added to the trial just before sowing and was not included in the seed measurements nor this picture. Accession 13 is pink because it is treated (chemical used in conventional agriculture) but has a cream coloured seed coating (similar to accession 12).

Figure 12. Sementes Vivas cowpea seed collection (photo: Dylan Wallman).

Table 2. Quantitative seed results

Accession	100-seed weight (g)	Seed length (mm)	Seed width (mm)	Seed thickness (mm)
1	12.2	9.5	6	4
2	17.3	10	6.1	5.5
3	14	8	5.9	4.8
4	18	8.6	6.5	5
5	16.7	11.5	5.5	4.1
6	16.1	11.3	6	4
7	16.1	8.5	6.3	5
8	19.2	8.6	6.7	5.5
9	10.7	7	5.2	4.9
10	21.8	10	7	5.7
11	23.7	11	7	6
12	23.6	10.5	7	6.1
13	12.4	8.4	5.8	4.5
14	13.8	8.8	6	5
15	13.2	7.8	5.7	4.3
16	15.8	11.5	6	4
17	12.6	10.1	5.9	3.7
.18	15	11.5	5.6	4
19	18	9	7	5
20	21.5	8.8	7	7.3
21	22.2	8.7	7.5	6.5
22	19.8	10.2	6	5.5
23	23.8	11	6.8	5.7
24	29.2	11	7.5	6.5
25	18.1	8.5	6.3	5.1
26	13.6	7.8	5.5	4.7
27	18	8.5	6.3	5
28	16	8,5	6	5
29	20.8	9.5	6	5.5
30	24.1	11.4	7.1	5
31	17.6	12	5.9	4
32	20	9.1	6.4	5.1
33	20	9.6	6.1	5
34	24	11.1	6.5	5.3
35	28.6	9.1	6.1	4.9
36	22.1	9.7	6.3	5.1
37	20	9.5	6.2	5.1
38	13.5	9.8	5.9	4.2
Mean	18.5	9.6	6.3	5.0

Table 3a. Qualitative seed results

Acc.	Coat colour	Eye colour	Eye pattern
1	Black	Absent	Absent
2	Brown	Absent	Absent
3	Cream	Black (some are brown)	Large around hilum
4	Cream	Brown	Thin around hilum
5	Brown	Absent	Absent
6	Brown	Absent	Absent
7	Cream	Beige	Medium around hilum
8	Cream	Brown	Thin around hilum
9	Cream	Light green/yellow	Thin around hilum
10	Cream	Black (some are brown)	Large around hilum
11	Cream	Black (some are dark brown, light brown, purple)	Large around hilum
12	Cream	Black (some are brown)	Large around hilum
13	Cream	Black	Medium around hilum
14	Cream	Black (some are brown)	Large around hilum
15	Cream	Black (silver in the end)	Medium around hilum
16	Brown	Absent	Absent
17	Black	Absent	Absent
18	Brown	Absent	Absent
19	Cream	Pink/purple	Medium around hilum
20	Brown	Slightly darker brown	Medium around hilum
21	Brown	Absent	Absent
22	Cream	Brown (some are dark brown)	Large around hilum
23	Cream	Brown	Large around hilum
24	Cream	Black (some are brown)	Large around hilum
25	Brown	Slightly darker brown	Medium around hilum
26	Cream	Light green/yellow	Thin around hilum
27	Cream	Brown	Thin around hilum
28	Black	Absent	Absent
29	Cream	Beige	Medium around hilum
30	Cream	Black (some are brown)	Large around hilum
31	Brown	Absent	Absent
32	Cream	Brown/orange	Medium around hilum
33	Cream	Black	Non-uniform (half the seed)
34	Cream	Black	Large around hilum
35	Cream	Brown/orange	Cover half the seed
36	Cream	Beige	Cover half the seed
37	Cream	Beige/orange	Medium around hilum
38	Cream & Brown	Black	Thin around hilum

Table 3b. Qualitative seed results (continuation)

Acc.	Seed shape	Testa texture
1	Kidney	Smooth
2	Rhomboid-Ovoid	Smooth to rough
3	Globose-Kidney	Smooth to rough
4	Ovoid	Smooth (almost shining)
5	Kidney	Reticulation (horizontal)
6	Kidney	Reticulation (horizontal)
7	Rhomboid-Ovoid	Smooth to rough
8	Ovoid	Smooth (almost shining)
9	Ovoid	Smooth to rough
10	Rhomboid	Smooth to rough
11	Rhomboid-Kidney	Smooth to rough
12	Rhomboid	Smooth to rough
13	Rhomboid	Smooth to rough
14	Rhomboid	Smooth to rough
15	Rhomboid-Elliptic	Smooth to rough
16	Kidney	Reticulation (horizontal)
17	Kidney	Reticulation (horizontal)
18	Kidney	Reticulation (horizontal)
19	Globose-Kidney	Rough
20	Crowder	Smooth
21	Crowder	Smooth
22	Rhomboid-Kidney	Smooth to rough
23	Kidney	Smooth to rough
24	Rhomboid-Kidney	Smooth to rough
25	Rhomboid-Elliptic	Smooth to rough
26	Ovoid	Smooth to rough
27	Ovoid	Smooth (almost shining)
28	Ovoid	Smooth
29	Rhomboid	Smooth to rough
30	Rhomboid-Kidney	Smooth to rough
31	Kidney	Reticulation (horizontal)
32	Kidney-Elliptic	Smooth to rough
33	Rhomboid-Kidney	Smooth to rough
34	Rhomboid-Kidney	Smooth to rough
35	Rhomboid-Ovoid	Smooth to rough
36	Rhomboid-Ovoid	Smooth to rough
37	Rhomboid-Ovoid	Smooth
38	Kidney-Ovoid	Smooth

Quantitative results

The collected data presented with the mean value of each accession for petiole length, petiolule length, terminal leaflet length, terminal leaflet width, length of main stem, and number of main branches are presented in separate tables in which the accessions are ordered by highest to lowest value. Letter grouping (Fisher's LSD) has been used to visualise which accessions that differ from each other significantly (Table. 4 – 9).

Table 4. Petiole length

Accession	Mean (mm)	Grouping
20	111.8	A
21	111.6	A B
8	109.8	A B C
16	109.2	A B C
14	108.8	A B C D
12	108.8	A B C D
5	105.8	A B C D
2	105.6	A B C D E
18	101.2	A B C D E F G
6	99.0	A B C D E F G H
9	98.4	A B C D E F G H
30	97.6	A B C D E F G H
19	97.2	A B C D E F G H I
27	97.0	A B C D E F G H I
4	96.8	A B C D E F G H I J
3	96.4	A B C D E F G H I J
10	96.0	A B C D E F G H I J K
7	95.8	A B C D E F G H I J K
32	95.4	B C D E F G H I J K
15	94.4	C D E F G H I J K
1	94.4	C D E F G H I J K
39	93.8	C D E F G H I J K L
17	93.8	C D E F G H I J K L
11	93.6	C D E F G H I J K L
29	92.8	D E F G H I J K L
24	92.8	D E F G H I J K L
28	90.4	E F G H I J K L
37	90.0	E F G H I J K L
36	89.4	F G H I J K L
22	89.4	F G H I J K L
13	86.8	G H I J K L
34	86.8	G H I J K L
25	86.0	G H I J K L
33	83.0	H I J K L
38	81.0	I J K L
35	80.5	J K L
23	80.0	K L
26	77.6	L
F-value	2.24	
LSD(0.05)	23	

Table 5. Petiolule length

Accession	Mean (mm)	Grouping
5	31.4	A
6	31.0	A B
18	30.6	A B C
21	28.8	A B C D
16	27.0	A B C D E
30	26.0	B C D E F
17	25.8	C D E F
34	24.8	D E F G
11	24.6	D E F G
29	24.4	D E F G H
10	24.0	D E F G H I
8	24.0	D E F G H I
37	23.4	E F G H I J
27	23.4	E F G H I J
24	23.4	E F G H I J
39	23.0	E F G H I J
25	23.0	E F G H I J
3	23.0	E F G H I J
32	22.8	E F G H I J
13	22.8	E F G H I J
1	22.8	E F G H I J
15	22.6	E F G H I J
22	22.4	E F G H I J
36	22.2	E F G H I J
12	22.2	E F G H I J
4	21.8	F G H I J
23	21.6	F G H I J
7	21.4	F G H I J
33	21.0	F G H I J
14	21.0	F G H I J
38	20.6	G H I J
35	20.4	G H I J
28	19.8	G H I J
26	19.4	H I J
20	19.2	I J
19	18.8	J K
2	18.4	J K
9	14.0	K
F-value	3.61	
LSD (0.05)	7.24	

Table 6. Terminal leaflet length

Accession	Mean (mm)	Grouping
5	133.6	A
6	133.2	A
18	132.2	A
21	127.4	A B
20	127.0	A B
17	124.2	A B C
16	121.8	A B C D
3	113.4	B C D E
36	109.6	C D E F
12	109.6	C D E F
35	109.4	C D E F
30	109.2	C D E F
25	108.2	D E F
8	108.2	D E F
32	107.4	D E F
24	106.2	D E F G
39	105.4	E F G H
1	104.4	E F G H
27	104.2	E F G H
22	103.8	E F G H I
29	102.4	E F G H I J
10	102.2	E F G H I J
4	102.0	E F G H I J
37	99.8	E F G H I J
33	99.4	E F G H I J
23	98.8	E F G H I J
19	95.4	F G H I J K
7	95.2	F G H I J K
28	95.0	F G H I J K
34	94.8	F G H I J K
14	94.2	F G H I J K
26	90.4	G H I J K
2	90.4	G H I J K
9	89.6	H I J K L
13	88.2	I J K L
15	87.4	J K L
38	82.8	K L
11	74.4	L
F-value	6.19	
LSD(0.05)	22.6	

Table 7. Terminal leaflet width

Accession	Mean (mm)	Grouping
35	75.6	A
5	75.4	A B
30	73.6	A B C
6	71.0	A B C
24	70.2	A B C D
25	68.8	A B C D E
21	68.8	A B C D E
39	68.6	A B C D E
12	68.2	A B C D E F
32	67.8	A B C D E F G
34	67.6	A B C D E F G
37	66.6	A B C D E F G H
16	66.2	A B C D E F G H
36	65.8	B C D E F G H I
17	65.8	B C D E F G H I
23	65.4	C D E F G H I
22	65.2	C D E F G H I
33	65.0	C D E F G H I
29	65.0	C D E F G H I
18	65.0	C D E F G H I
11	64.8	C D E F G H I
7	64.2	C D E F G H I J
28	61.0	D E F G H I J K
3	60.6	D E F G H I J K
20	59.4	E F G H I J K L
27	59.2	E F G H I J K L
19	58.6	F G H I J K L
13	58.2	G H I J K L
15	57.6	H I J K L
2	57.0	H I J K L
10	56.4	I J K L
14	55.0	J K L M
8	55.0	J K L M
4	54.6	J K L M
26	54.2	K L M
9	50.2	L M
38	49.8	L M
1	46.2	M
F-value	4.23	
LSD(0.05)	13.62	

Table 8. Length of main stem

Accession	Mean (cm)	Grouping
6	220.6	A
25	191.4	A B
17	182.8	A B
11	180.0	A B
38	176.0	A B C
30	167.6	B C D
28	157.4	B C D E
22	149.0	B C D E F
26	148.4	B C D E F
20	131.4	C D E F G
24	121.0	D E F G H
23	120.6	D E F G H
16	115.4	E F G H
15	111.6	E F G H I
39	110.2	E F G H I
21	109.8	F G H I J
33	108.2	F G H I J K
12	100.6	G H I J K L
13	97.4	G H I J K L
36	97.0	G H I J K L
3	92.6	G H I J K L M
9	89.8	G H I J K L M
4	84.0	H I J K L M N
8	83.4	H I J K L M N
27	82.6	H I J K L M N
10	76.8	H I J K L M N O
34	75.8	H I J K L M N O
7	73.8	H I J K L M N O P
14	67.2	I J K L M N O P
32	62.6	J K L M N O P
35	61.8	K L M N O P
1	60.6	L M N O P
29	59.2	L M N O P
19	48.4	M N O P
37	41.2	N O P
18	33.2	O P
2	28.0	P
5	26.8	P
F-value	8.23	
LSD(0.05)	67	

Table 9. Number of main branches

Accession	Mean	Grouping
25	10.2	A
26	8.8	A B
24	8.0	B C
19	7.6	B C D
12	7.6	B C D
10	7.6	B C D
11	7.4	B C D E
30	7.0	C D E F
28	7.0	C D E F
23	6.6	C D E F G
22	6.6	C D E F G
9	6.6	C D E F G
14	6.2	D E F G H
18	5.8	E F G H I
2	5.8	E F G H I
5	5.4	F G H I J
32	5.2	G H I J K
29	5.2	G H I J K
34	5.0	G H I J K L
21	5.0	G H I J K L
39	4.8	H I J K L
15	4.6	H I J K L M
36	4.4	I J K L M N
33	4.4	I J K L M N
13	4.4	I J K L M N
37	4.2	I J K L M N
20	4.2	I J K L M N
3	4.2	I J K L M N
35	4.0	J K L M N
1	4.0	J K L M N
27	3.8	J K L M N
7	3.6	K L M N O
8	3.4	L M N O P
38	3.0	M N O P Q
4	2.8	N O P Q
17	2.0	O P Q
6	1.8	P Q
16	1.6	Q
F-value	9.85	
LSD(0.05)	2.48	

Qualitative Results

The qualitative results are presented in table. 10 - 11 with figure. 13 as an extra resource to visualise the flower colour results.

Table 10. Vegetative trait results

Acc.	Plant vigour	Growth habit (1st)	Growth habit (2nd)	Terminal leaflet shape	Twinning tendency	Leaf colour
1	Low	Erect	Spreading	Hestate	Slight	Intermediate green
2	Medium	Erect	Bush	Sub-glubose	None	Intermediate green
3	Low	Intermediate	Spreading	Hestate	Intermediate	Dark green
4	Low	Erect	Bush	Sub-hestate	Slight	Dark green
5	Medium	Semi-erect	Bush	Sub-hestate	Slight	Dark green
6	High	Climbing	Climbing	Hestate	Pronounced	Pale green
7	Medium	Erect	Bush	Sub-hestate	None	Dark green
8	Low	Erect	Bush	Sub-hestate	Slight	Dark green
9	Medium	Erect	Spreading	Sub-hestate	Intermediate	Dark green
10	Medium	Erect	Bush	Sub-hestate	Intermediate	Dark green
11	Medium	Acute erect	Spreading	Sub-hestate	Pronounced	Dark green
12	Medium	Acute erect	Bush	Sub-hestate	Intermediate	Dark green
13	Medium	Erect	Bush	Sub-hestate	None	Dark green
14	Low	Erect	Spreading	Sub-glubose	Slight	Dark green
15	Medium	Erect	Bush	Sub-hestate	Slight	Dark green
16	Low	Climbing	Climbing	Hestate	Pronounced	Intermediate green
17	Low	Climbing	Climbing	Hestate	Pronounced	Pale green
18	Medium	Erect	Bush	Hestate	None	Intermediate green
19	Medium	Erect	Spreading	Sub-hestate	Slight	Dark green
20	Medium	Erect	Spreading	Sub-hestate	Pronounced	Dark green
21	Medium	Erect	Spreading	Sub-hestate	Pronounced	Dark green
22	Medium	Erect	Spreading	Sub-hestate	Pronounced	Dark green
23	Medium	Erect	Spreading	Sub-hestate	Pronounced	Dark green
24	Medium	Erect	Bush	Sub-hestate	Pronounced	Dark green
25	Medium	Semi-erect	Spreading	Sub-glubose	Intermediate	Dark green
26	Medium	Erect	Spreading	Sub-hestate	Pronounced	Dark green
27	Low	Erect	Bush	Sub-hestate	Slight	Dark green
28	High	Erect	Spreading	Sub-hestate	Pronounced	Dark green
29	High	Erect	Bush	Sub-hestate	Slight	Dark green
30	High	Semi-erect	Spreading	Sub-hestate	Pronounced	Dark green
31	n/a	Climbing	Climbing	Hestate	Pronounced	Dark green
32	High	Erect	Bush	Sub-hestate	Intermediate	Dark green
33	High	Erect	Bush	Sub-hestate	Intermediate	Dark green
34	Medium	Erect	Bush	Sub-hestate	Intermediate	Dark green
35	Medium	Semi-erect	Bush	Sub-hestate	Intermediate	Dark green
36	High	Semi-erect	Bush	Sub-hestate	Intermediate	Dark green
37	High	Erect	Bush	Sub-hestate	Intermediate	Dark green
38	Low	Climbing	Climbing	Hestate	Pronounced	Pale green
39	High	Erect	Bush	Sub-hestate	Intermediate	Dark green

Table 11. Reproductive trait results

Acc.	Date of first flower	Date of 50% flowering	Flower colour	Immature pod pigmentation
1	29-sep	Not reached	Violet	Not reached
2	25-sep	02-okt	Violet	Splashes of pigment
3	25-sep	Not reached		No pigmentation
4	01-okt	06-okt		Pigmented walls, green sutures
5	26-sep	08-okt	Violet	No pigmentation
6	16-sep	22-sep	Violet	No pigmentation
7	22-sep	10-okt	White	No pigmentation
8	30-sep	Not reached		Pigmented walls with green sutures
9	22-sep	Not reached	White	Not reached
10	21-sep	Not reached	White	Pigmented tips
11	19-sep	28-sep	White	Pigmented tips
12	19-sep	04-okt	White	Splashes of pigment
13	27-sep	29-sep	White	Pigmented tips
14	26-sep	Not reached		Pigmented tips
15	23-sep	04-okt	White	Pigmented tips
16	10-okt	Not reached		Not reached
17	22-sep	30-sep	White	Pigmented walls, green sutures
18	27-sep	Not reached		Not reached
19	01-okt	Not reached		Not reached
20	10-okt	Not reached		No pigmentation*
21	28-sep	Not reached	Violet	No pigmentation*
22	26-sep	29-sep	White	Irregular pigmentation*
23	19-sep	29-sep	White	No pigmentation*
24	21-sep	10-okt		Not reached
25	27-okt	Not reached		Not reached
26	29-sep	Not reached	White	Not reached
27	27-okt	Not reached		Not reached
28	16-sep	Not reached	White + Violet	Pigmented tips
29	19-sep	29-sep	White	No pigmentation
30	19-sep	22-sep	White	Pigmented tips
31	n/a	n/a	n/a	Red pods*
32	17-sep	22-sep	White	No pigmentation
33	19-sep	27-sep	White	Pigmented tips
34	19-sep	Not reached	White	Pigmented tips
35	19-sep	27-sep	White	No pigmentation
36	20-sep	25-sep	White	No pigmentation + pigmented tips
37	19-sep	22-sep	White	No pigmentation
38	28-sep	06-okt	White	No pigmentation
39	20-sep	27-sep	White + Violet	No pigmentation

***Result from the trial sown earlier in the season.**

Figure 13. Flower colours (photo: Dylan Wallman).

Discussion

General comments

Most of the accessions grown in this trial were unknown in terms of vegetative growth characteristics and reproductive factors. All the results were therefore of interest and served a purpose as an investigation of Sementes Vivas germplasm collection. Since the trial was conducted during the first year of the company's existence and no one working with it had any previous experience growing cowpea it also served as an initial trial just to get to know the crop itself. This section (Discussion) will include proposals for potential directions of future trials in order to develop cowpea cultivars for sustainable agriculture based on knowledge that has been gained during this process (field trials and literature review). It will also include analysis and reflections on the results that was obtained from the field trial.

When breeding for organic cultivation methods it is very important to incorporate resistance to pests and diseases to the new cultivars. Although it has been outside the scope of this thesis to investigate that aspect of cowpea production and breeding it is still worth stressing that work has to be done on this part. Information should be gathered on what pests and diseases that can threaten cowpea production in Portugal now and in the future. There are several germplasm sources with cultivars and breeding lines that contain resistance genes to several potential threats that can be used in breeding programs (see section 'Potential germplasm sources'). Identifying potential pests and diseases as well as collecting germplasm with the right resistance genes should be prioritised before or parallel to further cowpea trials and will most likely play a significant role in the success of future cultivars for both sole cropping and intercropping systems. When selecting or breeding cowpea cultivars for organic agriculture it could also be of extra importance to investigate and select for high nitrogen fixing rhizobia symbiosis efficiency since legumes can provide an organic source of nitrogen. This aspect was not included in this trial but could be good to incorporate at some stage of a future breeding or selection process.

This thesis together with other activities serves as start-up research on how to carry on with the project. At this moment the funds for the cowpea project are limited and there has not been any market analysis to investigate feasibility of a long term breeding program. Since the project is just starting up without an experienced cowpea breeder or researcher the workload could be expected to be higher during the first years when trial methods are being established. It is also important to set specific breeding objectives and prioritise the measurement of different traits in relation to them in order to design a cost efficient breeding program. It could also be beneficial to collaborate with research institutes and universities to investigate traits or conduct other types of research that require special methods or equipment.

Differentiation of Vegetable Cowpea (ssp. *sesquipedalis*)

Based on morphological characterisation (no genetic analysis) the germplasm collection of Sementes Vivas contains both the ssp. *unguiculata* (typical cowpea; commonly used for dried seed consumption) and ssp. *sesquipedalis* (vegetable cowpea; fresh pods used for cooking). Since these sub-groups normally differentiate in both growth habit and usage they could potentially be separated into different trial categories in the future. The harvest potential of vegetable cowpea should also be evaluated based on fresh pod weight and not dried seed weight which would normally be the case for typical cowpea. The ssp. *sesquipedalis* fresh pods are not commonly used in Portugal (personal observation, 2016) but might be interesting in terms of seed sales for home gardeners. During an open day at the Sementes Vivas farm these long pods (red and green) attracted a lot of positive attention from the visitors. Accession 6 which was one of the ssp. *sesquipedalis* varieties was also one of the earliest varieties to flower and set pods (although this was a late sowing; during the normal growing season other cultivars could potentially be faster). Although this accession had more vigorous plants and higher pod set in a trial conducted with similar treatment sown earlier the same season it is still one of the few accessions that managed to produce a decent amount of pods (based on eye observations and not included in the results) when sown late in the season. The accessions that belong to the ssp. *sesquipedalis* are 6, 16, 17, 31 (only grown in an earlier trial), and 38 that are climbing types as well as 5 and 18 that are bush types. The vegetable cowpea (ssp. *sesquipedalis)* with bush type growth habit originates from crosses between the ssp. *sesquipedalis* and the ssp. *unguiculata* (Acosta J.C. and Petrache I.M., 1960). They are primarily designed to reduce production costs since they do not need trailing that the climbing types need (Hall A.E. *et al.,* 1997). There has been no obvious usage of these bushy types of vegetable cowpea for Sementes Vivas at this stage of the project although little effort has been dedicated to investigate their potential and there might be associated benefits to this plant type.

Trial design discussion

The most important thing to notify is that the sowing date of the trial was late in relation to normal cowpea production in Portugal. Normal sowing time in Portugal for cowpea is in June and July (personal communication: Manuel Sousa Farnandes, 2016). This trial was sown August 5th and the earlier trial was sown June 14th. The small earlier trial conducted by an employee at Sementes Vivas showed differences in the development of the accessions common between the two trials; all the accessions of the early trial managed to develop completely and produce matured pods while most accessions in this trial stopped in development around flowering and only a few managed to set pods (and in those cases the pod setting ability were reduced). The delayed sowing led to certain limitations in what could be measured (e.g. yield) and reduced the possibility to evaluate the potential of the accessions for cultivation during normal growing season in Portugal. The trials were cancelled by November 1st without the possibility for the plants to reach full maturity (because of the seasonal decrease in temperature).

As mentioned in ‘Trial design and location’ the trial had one plot of each accession. It would have been preferred out of a scientific perspective to have at least three replications divided over three randomized blocks. This was unfortunately not possible for practical reasons such as amount of labour, uncertain space availability at the time of the trial planning, and (for some accessions) amount of seeds available. Since this initial trial was more qualitative and where the quantitative results will serve more as indications the decision to use one plot for each accession (and do pseudo-replications) could be argued to be justified.

The trial was irrigated during the first weeks of the trial (from sowing at the 5th of August and removed 13th of September). This trial was not planned to have irrigation but due to farm management practicalities this was the case during this time. How this initial irrigation impacted the development of the plants is not clear. Cowpea would normally be sown earlier in the season when there is slightly more rainfall (See figure 14: June) (personal communication: Manuel Sousa Fernandes, 2016) and the irrigation might have provided necessary water availability for the plants to have a decent survival rate during one of the warmest and driest months of the year in Idanha-a-Nova, Portugal (see figure 14: August). The early irrigation is not expected to have influenced the qualitative results of the trial. The main aspect that more likely could have been influenced is plant vigour (see 'Plant vigour' in the discussion section).

The weather data provided in the 'Trial design and location' in the material and method section comes from a website (World Weather Online, 2017) and is provided as an indication of the weather development and cannot be guaranteed to be completely accurate. Figure 14 represents a 30-year average from another weather website (Meteoblue, 2017) which could be used as a comparison and indication that the presented statistics could be representative.

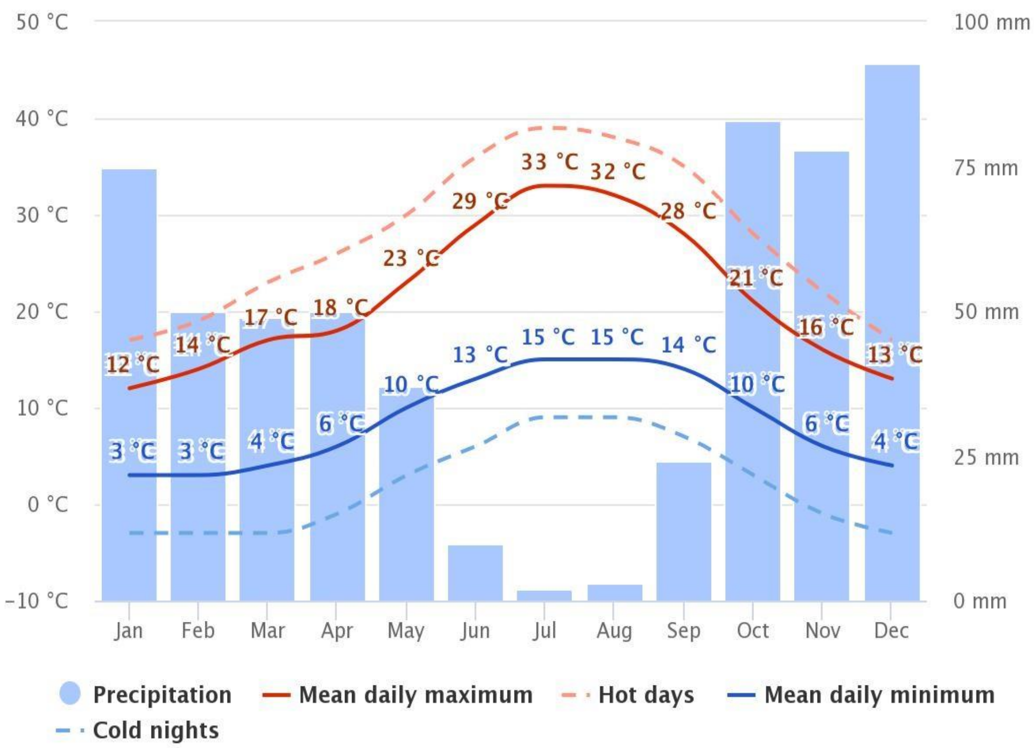

Figure 14. 30-year average weather for Idanha-a-Nova (source: Meteoblue, 2017).

It should also be mentioned that accession 31 was left out of the trial due to the lack of seeds. It has been included in the seed results and some qualitative results obtained from the small trial sown earlier in the season. Accession 39 is missing from the seed results because it was included in the trial just before sowing.

Trait discussion

Leaf related traits

The majority of the ssp. *unguiculata* accessions had dark green leaves while the ssp. *sesquipedalis* accessions had intermediate or pale green ones. Although it is not clear whether or not these colour differences is associated with chlorophyll content it might be worth mentioning that a low chlorophyll mutant managed to produce the same amount of average grain yield and biomass as its parent with normal chlorophyll content in a trial conducted over six years (Kirchhoff W.R. *et al.,* 1989). This study also mentions that many cowpea varieties actually have more chlorophyll than needed to maintain maximum photosynthesis levels during sunny day conditions. Hall A.E. *et al.* (1997) also refers to this and states that there seem to be no advantage in selecting for high chlorophyll per leaf unit area content.

Leaf shape was included in the trial for the sake of characterisation and no literature has been found that attributes significance to this trait for any breeding objective that would be interesting to Portugal or similar regions at this time. Pottorff M. *et al.* (2012) also states that there is no emphasis on breeding cowpea for the shape of their leaves and that leaf shape is mainly important for classifying and distinguishing cowpea varieties. It should also be mentioned that it was quite hard to distinguish between sub-hestate and sub-globose leaf shape and that the shape could slight variations within the same accessions (and plants).

Leaf size were assessed as ‘Terminal leaflet length’ and ‘Terminal leaflet width’ and are highly desirable for weed suppression ability (Hoad S.P. *et al.,* 2012) which makes them important traits to include when breeding or selecting cultivars for organic agriculture. The same source also gives importance (in relation to weed suppression) to the planophile leaf habit (leaf angle) which was not included in the trial but could be good to include in future trials. The highest values for terminal leaflet length were mainly obtained by the ssp. *sesquipedalis* accessions which also have the hestate leaf shape characterised by more long and narrow appearance. The value of big leaves in the ssp. *sesquipedalis* accessions might be limited in this context since they are not expected to be used by farmers I Portugal at this moment. It might on the other hand be possible to use these accessions to introduce the genes mapping longer leaves to typical cowpea cultivars. Accession 5 and 18 which are both bush types of ssp. *sesquipedalis* (which normally originates from crosses between the typical cowpea and vegetable cowpea (Acosta J.C. and Petrache I.M., 1960)) are two of the accessions with longest leaves – this might just be a coincidence but could also be an indication that this is trait can be transferred between the two subspecies.

No significance has been found in literature about the petiole and petiolule length but these traits could be assumed to also have some kind of significance related to weed suppression. Higher values of these traits could provide a wider plant canopy but might also allow more light to slip in between the leaves. The germplasm collection of Sementes Vivas showed significant differences for all these traits (terminal leaflet length, terminal leaflet width, petiole and petiolule length); all of these are data that could be useful when selecting breeding material for developing cultivars suited for organic agriculture. It should also be mentioned that these traits are quite time consuming to measure which can be good to take into consideration when planning upcoming trials and which traits to include.

Growth habit & other vegetative traits

The growth habit was evaluated twice with different methods. The first one was evaluated during the 6th week after sowing. The data from this evaluation was not sufficient in the sense that the growth habits expressed at this early stage changed later on during plant development. There are for instance accessions that were classified as erect in the beginning that turned out to be spreading later on. Since a lot of cowpea literature refers to spreading (prostrate) and bush (erect) growth habit it made sense to make an evaluation later on in the season distinguishing the accessions between bush, spreading, or climbing. The results from the early measurements are still included and should be seen as early stage growth habit rather than the appearance the plant had most of the growing season. It should also be mentioned that the later evaluation are rough estimation; there are variations between accessions that are classified as bush types as well as spreading types in terms of how spreading or bushy they actually are. There has not been any apparent need to make more detailed classifications of the growth habits at this stage of the project but it might be worth considering alternative methods for future trials. Ehlers J.D. and Hall A.E. (1997) mentions that cowpea cultivars are generally described at maturity as either erect, semi-erect, semi-spreading, or spreading (prostrate) which seem to be the most logical way to do it (assuming that climbing varieties are excluded from the trials). Unfortunately this categorization was discovered after the trials were conducted. Growth habit is an important trait that needs to be taken into consideration when choosing cultivars for different cropping systems. Hall A.E. *et al.* (1997) mentions spreading types to be more suited for intercropping systems while erect growth habit is generally preferred for sole cropping. A study comparing weed competitiveness between different growth habits found that erect growth habit is more competitive compared to semi-erect and spreading (prostrate) growth habit despite the fact that it had lower maximum photosynthetic rate and lower light use efficiency (Wang G. *et al.,* 2006). This can be good to take into consideration when breeding for weed competition and perhaps be combined with other weed competitive traits.

Twinning tendency has been included on a characterisation basis and no literature has been found that reviews the significance of this trait. In general seems to be important for the climbing ability; all the climbing cultivars have a pronounced twinning tendency, but how important this trait is for non-climbing cultivars is not clear. It could perhaps be interesting to investigate this traits impact on intercropping scenarios (for non-climbing types) and how twinning around the companion crop impact yield levels and harvest methods.

Length of main stem was included mainly for characterisation but can be assumed to play a role when selecting cultivars for different cultivation systems. Although no literature has been found that links length of main stem directly with weed suppression ability in cowpea it could be assumed to have a positive impact in the sense that a longer main stem could provide a taller or more spread out canopy that shades the area below. Even if weed suppression ability is an important aspect when breeding or selecting cultivars for organic agriculture it does not necessarily mean that a longer main stem always would be better - other factors (e.g. cultivation and harvest method) needs to be taken into consideration as well when deciding what type of length of the main stem that is required in a cultivar. Nelson C.S. and Robichaux R.H. (1997) mention that the number of nodes and internode length on cowpea is positively correlated to higher yields in intercropping systems. Number of nodes together with internode length on the main stem does not necessarily equal length of main stem in this context although it is more likely that a plant with a long main stem would score higher values on either or both number of these two factors. The accessions scoring high on length of main stem are either climbing or spreading types. It should be mentioned that since the trial were sown late in the season and the flowering stage were delayed or abstinent for some of the accessions it might have led to a bigger plant (due to a prolonged vegetative growth stage) compared to if it was grown during the normal cowpea season in Portugal.

Number of main branches was mainly included on characterisation basis but has been shown to be associated with yield in intercropping systems (Nelson C.S. and Robichaux R.H. 1997). Further discussion about these results can be found in the section 'Accessions interesting for intercropping trials' below. The number of main branches might in some accessions (as in the case of length of main stem) have been influenced by the prolonged vegetative stage caused by the late sowing date.

Plant vigour

The plant vigour was evaluated by eye observation dividing the accessions into three categories; low, medium, and high. These results only indicate the plant vigour in relation to the other accessions under the specific environmental conditions of the trial. Since the trial was conducted late in the year compared to typical cowpea production in Portugal these results might not be representative for how the accessions would perform if grown during the normal cowpea season. The trial was also watered during the first weeks (because of farm management practicalities) which might have influenced the plant vigour and favoured certain accessions more in relation to other ones. The method used differs from the standardized way of the IBPGR descriptor (IBPGR Secretariat, 1983) which use four categories based on actual size measurements. Since plant vigour is a very important trait when breeding for organic agriculture (early competition against weeds) (Hoad S.P. *et al.,* 2012) it is important to establish the most representative method for further trials. The method used in this trial might not be sufficient when selecting accessions for further development.

Days to flowering

The results for the days from sowing to flowering were not expected during the set-up of the trial and are not representative for cowpea production during the normal cowpea growing season in Portugal. As can be seen in table 11 almost half of the accessions did not even reach 50 % flowering. There was another cowpea trial conducted with similar treatments but sown on the 15th of June (this trial was sown 5th of August). Some of the accessions that barely managed to flower in this trial had a decent amount of flowers and also managed to produce mature pods in the earlier trial. There are some reasonable explanations for the abstinence or reduction in floral bud and pod development that occurred. Cowpea reproductive stages are induced and influenced by temperature and photoperiod (Hall A.E. *et al.,* 1997). Since cowpea is a short day crop and its level of photosensitivity probably is determined by multiple genes (Hall A.E., 1993; Ehlers J.D. and Hall A.E., 1996) it is likely that all or some of the accessions have some kind of photosensitive influence on their induction of flowers. Although photosensitivity is worth taking into consideration selecting cowpea cultivars for certain regions and cultivation practises, it is less likely that it is the cause of the delayed and reduced flowering in this trial. Since the days were even longer during the earlier trial and all the accessions there managed to produce flowers and mature pods it can be assumed that the short day photosensitivity were not the reason for delayed and reduced flowering, at least for the accessions that were common between the two trials. The more likely explanation is temperature related. Within certain limits warmer climate hasten the flowering in both photoperiod sensitive and photoperiod insensitive cultivars (Summerfield R.J. and Roberts E.H., 1985). In a trial conducted by (Ellis R.H. *et.al.,* 1994) the same accessions vary from around 35 - 45 days from sowing to flowering when cultivated with a mean temperature of around 29 ^{o}C to between 110 to 145 days from sowing to flowering when cultivated with a mean temperature of around 16 - 17 ^{o}C. Since the mean temperatures were sinking from around 23 to 16 ^{o}C in September and around 16 to 10 ^{o}C in October (see figure 6 in material and method section) it is possible that the time from sowing to flowering increase due to sinking temperatures within this period. That might also be the explanation why some accessions managed to start flowering but never reached the 50 % flowering stage. By the end of October the trials were cancelled with the conclusion that the plants would not develop much further due to the seasonal decrease in temperature.

Days from sowing to flowering can be important in relation to many different objectives. As mentioned in the section 'Breeding cowpea for intercropping' finding or developing cultivars that flower at a certain time in relation to the companion crop can be important to optimize the cowpea yield in intercropping systems. Days to flowering and days to maturation is significantly correlated (Udensi O. *et al.,* 2012) which indicates that work effort can be reduced by only measuring one of the traits. It is of course also important to have cultivar specific data for these traits in order to predict harvest times and select cultivar for different seasons, locations and production systems. There can be a lot of variation between different cultivars. There are cultivars that can flower 30 days after sowing and takes 25 days more to have mature pods while other cultivars can take up to 100 days to flower and between 210 and 240 days to reach full maturity (Singh S.R. and Rachie K.O., 1985). It can therefore be good to make trials during the normal cowpea growing season in Portugal to investigate days to flowering or days to mature pod formation, especially for the accessions already expected to have some sort of higher value in relation to different objectives.

Flower colour & Immature pod pigmentation

Flower colour and pod pigmentation was mainly included on a characterisation basis. The most important finding related to these traits was the fact that some accessions contained different flower colours which indicates that these accessions are not pure lines. The two accessions with different flower colours are number 28 and 39. The first mentioned one is black seeds separated from accession 27 which comes from a local gourmet shop in Castelo Branco (Portugal) and accession 39 comes from an unknown source; there were no expectations related to these accessions in relation to flower colour or genetic homogeneity but the results are good to know if any of these accessions turns out to have valuable traits for further development.

Not all accessions could be evaluated in terms of flower colour and immature pod pigmentation since some accessions never properly reached flowering or pod setting stage. Although all accessions managed to produce at least one flower some could not be evaluated on flower colour since the flower was discovered just before opening or when it had senesced without the possibility to re-evaluate it at the right time. The picture presenting the flower colours (see figure 13 in the section 'Qualitative results') should be interpreted with a bit of caution since the pictures were taken in different lights and the flowers look morphologically different because some of them were not fully developed at the time of the picture.

Some of the results for immature pod pigmentation come from the small trial that were sown earlier in the season which had some common accessions as this trial. These results are marked when presented. Immature pod pigmentation is a trait of interest for the ssp. *sesquipedalis* since they are produced for vegetable pod consumption. Most of these accessions had long green pods (some lighter and some darker green) but accession 31 which was not included in this trial (there was only enough seeds for the earlier sown trial) had red pods which gave them a very extravagant appearance (see figure 16 in section 'Similar and heterogeneous accession') that might be attractive for home gardeners and perhaps sold in smaller organic shops and niche markets (personal speculation).

Figure 15. Red vegetable cowpea (ssp. *sesquipedalis)* pod (accession 31) (photo: Dylan Wallman).

Seed Related Traits

The seed collection of Sementes Vivas has shown to contain seeds with 100-seed weight between 12,2 and 28,6 g. Hall A.E. *et al.* (1997) states that large seeded types with an individual seed weight above 200 mg (20 g of 100-seed weight) are preferred by consumers in many countries. Relatively large white (or cream) coloured, black eyed cowpea cultivars seem to be the standard type in Portugal (personal observation, 2016). The seed collection had several accessions that had the combined traits of high seed weight and the typical white coloured black eyed look. These cultivars could be used to incorporate these traits into new breeding lines although the option to use seeds with known pest and disease resistance as well as other agricultural aspects might save a lot of time and work. According to Hazra P. *et al.* (2007) the 100-seed weight also influences pod yield which might be good to take into consideration when breeding new cultivars.

It should be mentioned that the esthetical observations of the seeds (See table 3a and 3b) were conducted based on personal interpretations of descriptors and other research papers without the supervision of anyone with previous experience from cowpea seed characterisation. It is not expected that these results will play an important role on a detailed level at this stage of the project but if that would be the case a more thorough investigation is advised. It would also be advised to use seeds produced under equal treatment since different night temperatures has shown to produce different seed sizes (Hall A.E. *et al.,* 2003). The seeds measurements in this trial come from different production sites with different growing conditions since they were collected from multiple sources. This is also the reason why no statistical analysis has been conducted on these results – they are justified to include more as indications than absolute results.

Accessions interesting for intercropping trials

A few of the accessions in the trial has certain qualities that makes them good candidates for intercropping trials. All the recommended accessions have spreading growth habit since that is what has shown to be most successful in intercropping systems (Nelson C.S. and Robichaux R.H., 1997; Hall A.E. *et. al.,* 1997). Hall A.E. *et al.* (1997) also mentions that climbing types are of little use for intercropping systems. To limit the selection a bit further accessions that are not typical black eye or traditional Portuguese seed types has been excluded (since finding an accessions that functions well in intercropping systems but lacks a seed type acceptable to consumers would require breeding to introduce the right type of seed or a marketing campaign to make consumers accept the new seed type – both of these options are costly and assumed not to be viable compared to the option of first trying to find already intercrop-functioning cultivars with acceptable seed type to consumers). These limitations are mainly due to the assumption that any further cowpea and intercropping trials within the upcoming years will have very limited financial resources and should therefore be restricted to what can be assumed to have highest success rate according to reports from earlier studies on the subject. The accessions fulfilling these criteria are 11, 14, 19, 24, and 30 with typical black eye seeds and 9, 22, 23, 25, and 26 (9 and 26 are the same variety, see section 'Similar and heterogeneous accessions') with other types of traditional Portuguese seed types. Number of branches, number of nodes, and internode length is also associated with higher yields in intercropping systems (Nelson C.S. and Robichaux R.H., 1997). All the recommended accessions have relatively high values when it comes to number of main branches. Accessions 25 have the highest number of main branches of all the accessions in the trial with a mean value of 10.2 while the other accessions recommended for intercropping range between averages of 6.2 to 8.0 (see table 9 in results section). Number of nodes and internode length have not been assessed in this trial, but as mentioned in the section 'Growth habit & other vegetative traits' it is more likely that a plant with a longer main stem scores higher on either one or both of these traits. All the accessions recommended have relatively long lengths of the main stem except for accession 11 and 14 (which both originates from supermarkets in Portugal and can be assumed to be imported and produced in sole cropping systems). It should also be mentioned that accession 22 - 25 are traditional Portuguese varieties provided by INIAV and that cowpea seem to have been used for intercropping systems in Portugal in the past (personal communication: Jose Amorim, 2016). There could therefore be a possibility that these varieties actually have been selected through history to perform well in intercropping scenarios. It might also be a good idea to get in touch with other cowpea researchers to see if it is possible to obtain cultivars known to perform well in intercropping systems already.

Evaluating accessions for sole cropping

Although this trial was conducted in a sole cropping manner it has not provided results of some aspects that would be important to make a proper evaluation of in this kind of cropping system. It is important to define the target cultivation system when looking at what growth habits that would be preferred. If for instance mechanical harvesting will be applied accessions with erect growth habit and synchronous flowering and maturity is preferred (Ehlers J.D. and Hall A.E., 1997). At this stage of the project it is assumed that there is a limited set of resources in terms of time and money to make large scale trials of cowpea; it might be most efficient to set up quite strict criteria on what is wanted from a sole cropping cultivar and exclude all accessions that are known already not to fulfil the requirements. By doing this a properly replicated trial with a complete randomized block design can be conducted (without becoming too big and time consuming) to evaluate important aspects such as time to flowering, plant vigour, seed yield, and perhaps biomass production. If for instance Portuguese small to large scale organic farmers are the target group for the future seed sales it might be possible to exclude all accessions that does not have seed types that correlates to consumer preferences. If the project had more resources these accessions would also be interesting for further investigations to find out possibilities of using them for breeding purposes. Especially all the traditional Portuguese varieties would be of great interest to have proper data on yield and days to flowering. It should also be mentioned again that there has to be a proper investigation conducted on potential threats in terms of diseases and pests to actually perform any type of development or selection of cowpea varieties for organic agriculture (both for sole cropping and intercropping).

Similar and heterogeneous accessions

Some of the accessions of the Sementes Vivas cowpea germplasm collection turned out to be or at least seem to be the same varieties (based on morphological characterisation). There were also accessions that indicated heterogeneous genetic composition in the sense that they internally had diverse traits. This diverseness has mainly been detected because of different flower colours. There could be more accessions with diversity within the same accession that has not been discovered because their diversity has not been as obvious as diverse flower colours. The accessions that most obviously have been the same variety are 4, 8, and 27; these accessions all comes from different collection points sold for human consumption (probably imported from another country) but have the same plant type and identical seed types. Although not properly evaluated for this trial these three accessions were observed to have quite eye catching red pigmented stipules uncommon for any of the other accessions from the germplasm collection (see figure 16). Accessions 9 and 26 are also supposed to be the same. These two accessions have significant differences on some of the quantitative traits but since they are based on pseudo replications this should not be emphasised too much. These two accessions are a traditional Portuguese cowpea type called 'Bago de Arroz' or commonly just referred to as 'Arroz' (personal communication: Paulo Martinho, 2016). It should also be said that accession 32 – 37 are seeds from the same bag (also mentioned in the section 'Consumer preferences', see figure 2) from a farmers market and was separated into different accessions based on eye colour and eye shape for this trial. These accessions might have been grown together by a small scale farmer as a variety mixture (but could also have been added together from different sources before being brought to the market). The vegetative stage of the different accessions from this variety mixture was all similar and they all had white flower colour.

Figure 16. Red pigmented stipules of accession 4, 8, and 27 (photo: Dylan Wallman).

Two accessions, 28 and 39 were obviously genetically heterogeneous since they had different flower colours within the same accession (see figure 13 in the result section). The source of accession 39 is unknown while accessions 28 are black seeds separated from another accession from a local shop in Castelo Branco. This accession is interesting in the sense that it contained plants that either produced black or cream coloured seeds when all the seeds that were sown for this accession were black. No explanation has been found for this phenomenon except that it might be a result of outcrossing which can occur to a low but still significant level in breeding nurseries and seed production fields (Ehlers J.D. and Hall A.E., 1997).

Germplasm sufficiency for breeding

It is hard to make any assumptions on how suitable or sufficient the germplasm collection of Sementes Vivas is without further trials and investigations. Aspects such as pest and disease resistance (and tolerance) needs to be included and it would make sense to conduct some yield trials as well. This trial has never the less showed that there are a wide range of plant types (growth habit, length of main stem, number of branches, leaf size, etc.) within the collection. There are also seed types that correlate with the most common consumer preference (white or cream coloured with black eye – see section 'Consumer preferences') as well as some traditional Portuguese varieties. This thesis also mentions some institutions that might be able to provide information as well as germplasm material (see section 'Potential germplasm sources') that could be useful and resource saving. It might for instance be more feasible to get some varieties with known resistance to certain pests and diseases rather than to screen the entire germplasm collection to find out if it contains resistant accessions already.

Conclusion

The germplasm collection of Sementes Vivas turned out to contain varieties and traits that could be interesting for selection and breeding in different directions such as home gardens, intercropping and sole cropping. There is still work needed to be done to define more specific breeding goals, especially in terms of pest and disease resistance, but also financial aspects could be good to investigate in order to prioritise different objectives wisely. Some accessions (primarily the traditional Portuguese ones) could already be of interest to make further sole cropping trials to investigate yield potential and days from sowing to flowering. Intercropping trials are more complex since there are more factors involved and they require more space and resources to perform but there are accessions in the germplasm collection that could be good candidates for intercropping trials as well. It would also be recommended to separate the germplasm collection between typical cowpea (ssp. *unguiculata*) accessions and vegetable cowpea (ssp. *sesquipedalis*) accessions since they differentiate significantly in cultivation practise and crop usage. There are institutions with large germplasm collections that might be able to provide useful seeds with known plant characteristics that could be useful to include in upcoming trials and breeding projects. Finding and selecting already existing cultivars suited for the different cultivation systems would be a reasonable short term objective while breeding new cultivars as well as developing new cultivation systems should be considered as long term objectives. Listed below are some suggestions on ways to follow up this project in the upcoming years:

- Keep collecting germplasm material (with a priority on finding more traditional Portuguese varieties and cultivars with known heat tolerance as well as pest and disease resistance).
- Investigate current and potential pest and disease problems in Portugal as well as other target regions for seed sales.
- Divide further trials into different categories: home gardens, intercropping, and sole cropping.
- Select and prioritise the accessions for the different new trial categories based on the characterisation and information provided in this thesis.
- Perform home garden and sole cropping trials that investigates days to flowering and yield potential (as well as other aspects that can be useful in relation to seed sales) in order to market some varieties as soon as possible. Selection of accessions can be based on information provided in this thesis (traditional Portuguese varieties and cultivars with seed or pod types correlating with consumer preferences should be prioritised).
- Initiate research on the companion crops for the cowpea intercropping systems (what are the demands in relation to producers and the market as well as how different traits and plant characteristics of the companion crop influence the cowpea yield).

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Appendix

Farmer interview: small scale organic cowpea in Portallegre

12 May, 2016

The farming method by this farmer might not be representative for cowpea farming in Portugal and no research has been conducted within this thesis work to see if that is the case or not. Never the less it served as a knowledge base when the trials were planned and might be useful for future researchers working with the project. All the information comes from personal communication with the farmer himself (Manuel Sousa Fernandes).

The cowpea is sown in June or July and is cultivated without the use of any irrigation. If it is a hot year an exception is made and there will be one proper watering at the time of sowing. This farmer has good soils so his method might not be representative for all farmers although he claims that growing cowpea without irrigation is the standard practise all over Portugal. Before sowing two to three false seed beds and harrowing are done to reduce the amount of weeds. If the soil is dry at this stage it has to be irrigated. The seeds are put in water for two to three hours before sowing and they germinate within two to three days. The farmer sows the seeds by throwing them by hand and the field is not entered until it is time to harvest. The same field is used year by year and is cultivated with grass during the winter. He claims to have no pest problems although if the weather is wet there can be something he refers to as “plant flees”. When the seeds are sown by the end of June the pods can be harvested from the middle of September until November. The weather has to be dry in order to harvest the pods dry. Since the pods do not mature at the same time they have to be harvested by hand. A good harvester can harvest 6 kg an hour.

He also donated two cowpea varieties to the project. One is a green eyed type (accession 29) originally from Castelo Branco that traditionally has been sown after the harvest of other crops in June or July. The other one is a black eyed type (accession 30) originally from Cebolis (a small village near Castelo Branco). Both have a bushy growth habit and indeterminate growth pattern. He sells the harvest for around 3 euro/kg. According to him people prefer the green eyed type that they eat with rice. It does not make any sense for him to replace the cowpea crop with a maize and cowpea intercropping system since cowpea has a higher market price than maize.