ABSTRACT

Optimization of seed storage conditions in various legume species by analyzing abiotic factors

Keywords: seed, garden peas, cultivation, storage process optimization

Seed is a special agricultural product in two respects: as a source of food for humans and animals, but more importantly as a biological propagating material for crop establishment.

When it is used to establish new crops, the importance of the seed becomes major, as it is the carrier of the genetic characteristics of the original breeding material. The better these characteristics will be manifested, the better the seed will have a set of morphological, anatomical, physiological, chemical and biochemical characters and attributes which define, in short, the quality of the seed.

A quality seed is agronomically evaluated by certain indices such as physical purity, moisture, germination, health, vigor, etc.

The quality of the seed is determined by a whole chain of factors (objective or subjective), such as: the natural framework conditions, the work involved in establishing the crop, the maintenance work, the harvesting of the seed, its conditioning, the packaging, storage and marketing, on which the future harvest largely depends.

From the moment it is received, the seed acquires a particular biological value, which is attested by a quality certificate, and finally it acquires agronomic value, which ensures the economic efficiency and sustainability of the production activity.

For this reason, the PhD thesis aimed at optimizing the storage conditions of pea seeds through the evaluation of some morphological indicators (germination, radicle, hypocotyl), some primary and secondary metabolic compounds (proteins, lipids, total and digestive fibers, caloric energy, ash, etc) and some macro and microelements (N, K, Mg, Ca, Fe , Zn, Na) in four cultivars (Zsuzsi, Gloriosa, Meraviglia and Kelvedon Wonder), kept for three years (2017, 2018, 2019) under the influence of five different conditions of temperature and atmospheric humidity (t=4°C x U=8%; t=4°C x U=12%; t=8°C x U=8%; t=8°C x U=12%; t=22°C x U=65%).

In order to achieve the proposed aim, I set the following objectives:

- To evaluate the influence of storage conditions and seed age in pea on morphological indicators (germination, germination energy, radicle and hypocotyl length);
- evaluation of the influence of storage conditions and age of pea seeds on primary metabolite compounds (moisture, lipids, ash, protein, total fiber, dietary fiber, caloric value);
- evaluation of the influence of storage conditions and age of pea seeds on some macro and micro elements (N, K, Mg, Ca, Fe, Fe, Zn, Na);
- mathematical modeling on the optimization of pea seed storage conditions through the evaluation of morphological, physiological and biochemical indicators in Gloriosa and Kelvedon Wonder cultivars.

The proposed aims and objectives will provide pertinent answers on intimate (biological and biochemical) processes that will be of interest to both scientific researchers and, more importantly, practitioners in seed production units.

The studies and research were carried out within the framework of the doctoral studies program conducted at IOSUD of USV Iași, within the Doctoral School of Engineering Sciences, in the doctoral field Horticulture, specialization Legumiculture.

The research work was carried out in the field, at a commercial company that stores and commercializes seeds, the Discipline of Vegetable Cultivation, its research laboratory and at the biochemical analysis laboratory of USV Timișoara

The PhD thesis is structured in two parts:

- Part I - Current state of knowledge on the improvement of pea seed quality by some technological measures;

- Part II - Results of own research.

Part I of the PhD thesis is structured in three chapters and comprises: Chapter 1. The circumstances of legume seed production in Romania; Chapter 2. Seed conditioning and storage in Romania and Chapter 3. The current state of research on the optimization of seed storage regime in garden pea.

In the first two chapters of Part I, a synthesis of national and international knowledge on the importance of using quality seed for the establishment of commercial crops and seed production and the indicators that define this quality, as well as the technological flow that strictly concerns the seed as an object of study, is summarized. For the most part, this synthesis is based on the provisions of the Romanian and international legislation on seed, as well as on the most important information from the specialized literature in this field.

Chapter 3 presents studies on the effect of environmental conditions on the preservation of legume seeds; studies on the viability and physico-chemical composition of legume seeds; and studies on the factors leading to seed deterioration and measures that can be taken against loss of viability

Part II of the dissertation is presented in three rather comprehensive chapters and accounts for more than 2/3 of the total volume of the dissertation.

Chapter 4 - Research aims and objectives. The biological material used and the general methodology of work, has three sub-chapters: Aim of the research; Objectives of the research and Materials used and general methodology of work

Referring to the aim and objectives of the research topic, presented in a previous paragraph, in the text dedicated to this subchapter the need for this scientific approach is shown, based on the current circumstances related to the optimization of seed storage conditions and the need for an analysis of seed quality according to the diversity of cultivars used and the age of the seeds in relation to some morphological indicators, their chemical and biochemical composition.

The biological material used in the research was represented by four pea cultivars (Pisum sativum L.): Zsuzsi, Gloriosa, Meraviglia and Kelvedon Wonder.

Factor B was represented by crop year which had three gradations: b1-'2017'; b2-'2018'; b3-'2019'.

The C factor was determined by the storage conditions with five gradations, determined by the temperature and humidity in the storage space, as follows: c1-'SC1-t=4°C x U=8%'; c2-'SC2-t=4°C x U=12%'; c3-'SC3-t=8°C x U=8%'; c4-'SC4-t=8°C x U=12%'; c5-'SC5-t=22°C x U=65%' (Control).

The morpho-physiological indicators were determined in the USV Iași's vegetable growing laboratory and the chemical and biochemical analyses were carried out in the biochemistry laboratory of USV Timișoara.

The general work methodology comprised research packages:

- Case study that analyzed the technical-administrative conditions at a company dealing with seed storage and preservation under controlled conditions;

-germination study carried out on filter paper, 50 seeds in three repetitions. The method is described by Stoleru et al., 2019. The radicula was measured two days after the seeds germinated and at the end of

the experiment (on the fifth day). Hypocotyl length was measured on the fifth day after seeds germinated; radicle and hypocotyl length were measured with an electronic caliper.

-The primary compounds that were analyzed in pea seeds were: moisture, lipids, crude protein, crude protein, crude fiber, dietary fiber, free N and caloric value.

-macroelements determined were: calcium, magnesium, potassium and sodium and microelements determined were: iron and zinc, using ICP-MS with a Nexion 300D quadruple mass spectrometer.

The study of the technological flow of storage summarizes the main elements of the technological flow of storage, storage and delivery, including reception, date, quantities, packaging, type, number of packages, location in the space, verification of microclimate conditions, sampling, number of samples, species, controls, control of environmental factors, packaging, repacking, preparation for delivery, delivery.

In conclusion, it can be said that the Remington Seeds Romania seed sorting station in the county of Iași has all the environmental conditions necessary for sorting, storing and checking seeds in good conditions. The technological flow is established by a program of activities that complies with the legislation on seed storage, packaging and movement.

Chapter 5, which is the most comprehensive, deals with the influence of storage conditions on morphological, physiological and biochemical indicators in pea seeds. This chapter presents the results for the four cultivars on the influence of pea seed storage conditions and year of harvest on: germination, germination energy, radicle and hypocotyl length, seed moisture, primary and secondary metabolism compounds, or some minerals such as: Free N, K, Mg, Ca, Fe Zn, Na, elmenete which are involved in the metabolic stress of their storage in the stores.

Storage conditions of pea seeds significantly influence their germination and germination energy. For garden pea seeds stored in the period 2017-2019 it can be observed, that based on the analyses performed there are significant differences depending on cultivar and storage conditions. Thus, it can be seen that regardless of storage conditions, seed germination meets the quality conditions for the 2019 seed to be marketed, regardless of cultivar .

Higher values of germination and germination energy are recorded for the 2019 seed and cultivar KW. The data show that the 2019 pea seed can be stored under both normal and controlled conditions. Storage for periods longer than 1 year is better under controlled conditions as the germination percentage is higher.

The radicle length varied in quite wide range from 10.71 mm in the case of cultivar Gloriosa harvested in 2017, seed stored under normal conditions of temperature and humidity and 39.43 mm in the case of seed harvested in 2019 stored at 4°C temperature and 8% humidity, the difference was 368.16%, which denotes that there are highly significant differences between radicle length determined by cultivar, storage conditions and age of seed.

The hypocotyl length ranged within quite wide limits, from 2.51 mm in the case of cultivar Gloriosa harvested in 2017, seed stored under normal temperature and humidity conditions and 14.39 mm in the case of seed harvested in 2019 seed stored at 4°C temperature and 8% humidity, the difference being 573%, which denotes that there are highly significant differences between hypocotyl length determined by cultivar, storage conditions and seed age.

Significantly higher results in radicle and hypocotyl for lower seed age has a favorable effect on future plant vigor with favorable effects on yield capacity.

Seed moisture together with temperature are the most important abiotic factors on which seed vitality depends. Under high temperature and moisture conditions, seed vigor decreases or even the future of the crop may be in question.

The data obtained show that moisture was more influenced by storage conditions than by cultivar, as it is well known that at harvest and storage for storage, seed should have a moisture content of 11-13%. For all cultivars, seeds were placed in storage chambers at 11 ±0.01% moisture.

Moisture varied within fairly wide limits, ranging from 11.23% for Kelvedon Wonder cultivar harvested in 2019 and stored at 4°C temperature and 8% humidity and 15.13% for Zsuzi seeds harvested in 2017 stored under normal temperature and humidity conditions, the difference being 25.77%.

The lipid content varied within quite wide limits, from 23.37 g/100 g s.u. in the case of Gloriosa cultivar harvested in 2017 and stored under normal atmospheric conditions to 31.86 g/100 g s.u. in the case of Zsuzi cultivar harvested in 2019 and stored under optimal temperature and humidity conditions, the difference of 36.33% being highly statistically significant ensured.

Significantly better results were obtained especially in the case of low-age seeds, which means that they will have a net favorable effect on future crops.

Peas have a high potential to accelerate the dietary transition due to the high protein content of the seeds and the relatively well balanced amino acid composition. Under storage conditions, the seed metabolism is altered, therefore it is likely that aging reactions are randomly influenced.

The analyzed data show that protein is influenced by both storage conditions and age. In the case of three-year-old seeds, a slightly higher protein content is found, but the differences are insignificant.

The protein content ranged within low limits, from 26.49 g/100 g s.u. in the case of cultivar Meraviglia harvested in 2019 and stored under normal atmospheric conditions to 31.11 g/100 g s.u. in the case of cultivar Zsuzi harvested in 2017 and stored under optimal temperature and humidity conditions. Higher values are observed especially in 3-year-old seeds, which means that plant vigor is not determined by higher protein content.

Under storage conditions, seed metabolism is altered, therefore it is likely that aging reactions are randomly influenced by internal or external factors.

Total fiber content is influenced by both storage conditions and age. Slightly higher total fiber content is found in three-year-old seeds, but the differences are insignificant.

Nutritive value is the ability of food to meet the physiological requirements of the body. Calorific value is the energy provided by the components of the food that have an energetic role (free N, ash, carbohydrates). Under the given conditions, the caloric value of pea seeds is influenced by both storage conditions and their age. In three years old seeds, a lower value is found, the differences are significant for $p \leq 0.05$. The caloric value varied within low limits of about 13%, ranging from 474 kcal/100 g in the case of cultivar Gloriosa harvested in 2017 and stored under normal atmospheric conditions to 540 kcal/100 g in the case of cultivar Zsusi harvested in 2019 and stored under optimal temperature and humidity conditions.

The amount of ash, although a gross parameter, is an indicator of the mineral content of the edible part of the kernel, which includes elements essential for human life; some of these mineral elements include Fe, Zn, Mn and Cu. Pea seeds are used as food worldwide, and improving the mineral content of dry pea is indispensable to combat a wide variety of health problems associated with mineral deficiencies. With proper storage, the depreciability of the seeds decreases, and therefore mineral loss is reduced.

The obtained data reveal that ash varies depending on the age of the seeds, cultivars used and storage conditions. The ash content varied within quite low limits, from 3.62 g/100 g s.u. in the case of Zsuzi cultivar harvested in 2017 and stored under normal atmospheric conditions to 4.72 g/100 g s.u. in the case of Meraviglia cultivar harvested in 2019 and stored under optimal temperature and humidity conditions, the difference of 22.6% % being significant. In the case of Meraviglia cultivar, due to its high

mineral content, it is indicated in case the germination decreases to be immediately released for consumption due to its high quality in secondary metabolism compounds. Higher values of the ash content are observed especially in the case of low-aged seeds, which means that with the age of the seeds the mineral content also decreases, but the decreases are much smaller than in other parameters.

Seed age is an important biological factor on which seed vigor depends. In general, older seeds lose vigor rapidly due mostly to primary metabolic compounds.

The free N content varied within small limits, from 41.77 g/100 g s.u.s. for cultivar Zsuzi harvested in 2017 and stored under conditions of 22°C and 65% humidity to 47.89 g/100 g s.u.s. for cultivar Meraviglia harvested in 2019 and stored under conditions of 4°C and 8% humidity, the differences being 14.65%, statistically insignificant.

The K dynamics in pea seeds is mainly influenced by storage conditions and less by cultivar. In three years old seeds, a lower content is observed, the differences being insignificant for $p \leq 0.05$. During the storage period, K losses are not significant, but on the contrary depending on cultivar and conditions the values are even higher, without this mineral element obviously influencing the germination of pea seeds, compared for example with free N, which obviously influences seed germination.

The data obtained for Mg highlight the biological value of the cultivar and storage conditions in terms of the content of this element, obviously influencing morphological and physiological parameters in young plants; for certain cultivars and under certain storage conditions, Mg losses are not statistically significant.

The conditions of 4°C and 12% humidity caused the values of Ca content in seeds to vary between 562.40 mg/kg d.w. in Gloriosa harvested in 2019 and 595.57 mg/kg d.w. in Zsusi harvested in 2019, which denotes that the values for these seed storage conditions do not depend on their age.

In the case of Fe, it is found that its dynamics depend on the biological value of the cultivar and the storage conditions in terms of the content of this element, which positively influences the morphological and physiological parameters of the seeds.

The recorded values highlight reduced losses in Na, which means a much lower degradation under storage conditions. However, the lowest values in Na are recorded when they were stored at a temperature of 22°C and a humidity of 65% with lower values in the Meraviglia and KW varieties.

Based on the results obtained in the previous chapter, mathematical modeling is used to optimize pea seed storage processes for two of the four cultivars, namely Gloriosa and Kelvedon Wonder.

The present study confirms that seed storage management, depending on temperature and humidity, can significantly influence the main morphological indicators that determine the vigor and implicitly the productivity of the cultivar. The finding that there were variations in seed germination with age indicates that the seed preservation method has profound statistical significance and can positively influence the quantity and quality of the harvest.

Seed storage conditions and the year of harvest have a particular importance on the primary and secondary metabolism compounds in pea seeds. It has been demonstrated that the temperature of 4°C and the humidity of 8% have a positive effect to extend the preservation of seeds for the main parameters of germination and secondary metabolism compounds in pea seeds for two years, creating the possibility of producing legume seeds every two years, which is currently not allowed by national and European legislation.

Biochemical parameters of seeds are directly influenced by age and storage conditions. Seed age had a statistically significant influence on ash and free N content of pea seeds.

The content of macro and microelements in pea seeds was directly influenced by storage conditions and harvest year. In the case of macroelements, the trend was: K>Mg>Ca>Na, while for microelements it was: Fe>Zn. The significant variations of macro and microelements in seeds of the Gloriosa variety compared to KW showed that during the storage period there is a greater ecological plasticity, regardless of storage conditions and therefore a better adaptation to crop conditions.

Current research has highlighted the continuous need to perform interdisciplinary, morphological, chemical, biochemical, microbiological and physiological analyses in order to achieve correlations with biochemical factors of deterioration, and especially with enzymatic activity.

Based on the results obtained, general conclusions and synthetic recommendations were drawn, from which it is clear that the purpose and objectives of the thesis were fully achieved.