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Final Activity Report

Lathyrus diversity: available resources with relevance to crop improvement – progress reviews and relationship with AEGIS

(EUGrainLeg)

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INTRODUCTION

The Grain Legumes Working Group of the European Cooperative Programme for Plant Genetic Resources (ECPGR) covers a range of species that have enormous benefits for the environment, food security as well as breeding material. The existing germplasm, including *Lathyrus* spp., has huge potential to be exploited for multiple purposes.

***Aims**:* The EuGrainLeg Activity was a contribution of the Grain Legumes ECPGR Working Group (WG) to meet the ECPGR objectives for phase IX (2014–2018). The Activity objectives were (1) to ensure AEGIS progress by improvement of *Lathyrus* data characterization and introduction of *Lathyrus* accessions featured by improved quality and quantity of phenotypic data in the European collections, (2) to ensure: visibility via different presentations/ publications and accessibility for potential users of *Lathyrus* genetic resources (GR) via the EURISCO database, (3) to facilitate interaction and knowledge exchange in ECPGR's Grain Legumes WG.

Background: the Activity was developed after SMARTLEG's implementation. This Activity was previously granted in the frame of the Third Call of the ECPGR Activity Grant Scheme and brought progress towards Objectives 1 and 2 of Phase IX. SMARTLEG was finalized with new phenological, morphological and molecular data, available to EURISCO and recommended for AEGIS.
Considering the need for existing available GR and the importance of well-described data, members of the Grain Legumes WG, decided to focus on grass pea (*Lathyrus sativus* L.) thanks to its robustness, resilience to climate change and potential to provide survival food during drought-triggered famines (Lambein, 2019). Moreover, grass pea has a huge versatility being used for a variety of purposes, such as animal feed and fodder (Rizvi et al. 2016), but also as human food, thanks to 18–34% and 17% of protein content in seeds and mature leaves and 58% of polyunsaturated fatty acids (Grela et al. 2010).
The status of *Lathyrus* species is variable, in some countries (partners in EuGrainLeg) being poorly represented (Romania), considered neglected and very little exploited in agri-food chains; in other countries, many accessions are available, for example, Bulgarian and Greek landraces are being used for human consumption. In Portugal, 21 *Lathyrus* species occur, the majority being wild or neglected species. *Lathyrus sativus* L., *Lathyrus annua* L., *L. cicera* L. and *L. odoratus* L. were used for food, fodder or ornamental species (Vaz, Patto & Rubiales, 2014). Grass pea landraces were used for human consumption in Portugal's mainland and Madeira, with more than 200 accessions conserved in germplasm banks. Sinkovič et al. (2020) mentioned that the local wild and agricultural floras in Slovenia and Bosnia and Herzegovina are relatively rich in *Lathyrus* spp. In these two countries, grass pea (*Lathyrus sativus* L.) is traditionally used as a grain for human consumption and its foliage is used for fodder (Gatarić et al., 2009; Mikić et al., 2011; Kosev & Vasileva, 2019; Kosev et al. 2019; Vasić et al., 2019). A variable number of available accessions for different species were identified.

***The challenge*:** to acquire useful information for end-users (e.g. breeders) by strengthened collaboration between ECPGR members/institutes.

***Expected outcomes**:* increased number of AEGIS *Lathyrus* spp. accessions, morphological and agronomical data on a selected set of European *Lathyrus* spp., and increased quality and quantity of data in EURISCO.

***List of involved partners and their respective roles*:**

- Funded members from Romania, Bulgaria, Portugal, Greece, Serbia, Slovenia, Bosnia Herzegovina (Bosnia Herzegovina and UK were not available to participate in the workshop). Except for the UK, the rest of participants exchanged seeds, decided on a working plan and carried out field experiments for phenological investigations.
- Self-funded members from Romania, Serbia, Russia (Russia was not available to participate in the workshop, or to be involved in further experiments).
- Invited observers at the workshop in Bucharest 2019: IPK Germany, ICARDA, UNICT – Italy coordinator of BRESOV H2020 project, ASAS Bucharest Romania – local organizer.

MATERIALS AND METHODS / APPROACH

The work plan of the Activity was developed based on the approach outlined in figure 1:

- Action 1: Pre-workshop actions: inventory of available GR of *Lathyrus* national collections, to identify, propose and provide materials to be investigated in the frame of EUGrainLeg.
- Action 2: Workshop (in-person meeting): partners analyzed the national collections of *Lathyrus*, and established a *Lathyrus* panel, based on exchanged materials.
- Action 3: Post-workshop actions: investigations towards the establishment of high-quality *Lathyrus* data according to the principles of AEGIS; Final meeting online to assess the progress and the accomplishment of the actions; Final report.

Material: A panel consisting of 26 accessions of *Lathyrus sativus* was developed according to available seeds from partner units.
Locations: Seven locations were established for phenotyping investigations in Bosnia and Herzegovina, Bulgaria, Greece, Portugal, Serbia, Slovenia, and Romania (Figure 2).
Methods: Phenotypical investigations, using common templates harmonized based on Descriptors for *Lathyrus* spp. (IPGRI, 2000).
The list of descriptors and methodology of data collection are presented in Table 2, Annex 2, and included traits related to: vegetative characters (seedling vigour and plant growth habit), stem characters (plant height [cm]), branch characters (number of primary branches), leaf characters (leaflet shape), flowering characters (days to first flowering [d], days to 50% flowering [d], days to maturity [d], flower colour), pod characters (pod-bearing position [cm], number of pods per plant, pod shape, pod length [cm], pod width [cm], number of seeds per pod, pod dehiscence), seed characters (shape, size, coat colour, 100-seed weight [g], seed yield per plant [g]), susceptibility to disease (visually assessed).
Phenotypic investigations were carried out as planned in 2019 and additionally as in-kind contributions in 2020 in some locations (Romania, Bulgaria and Serbia).

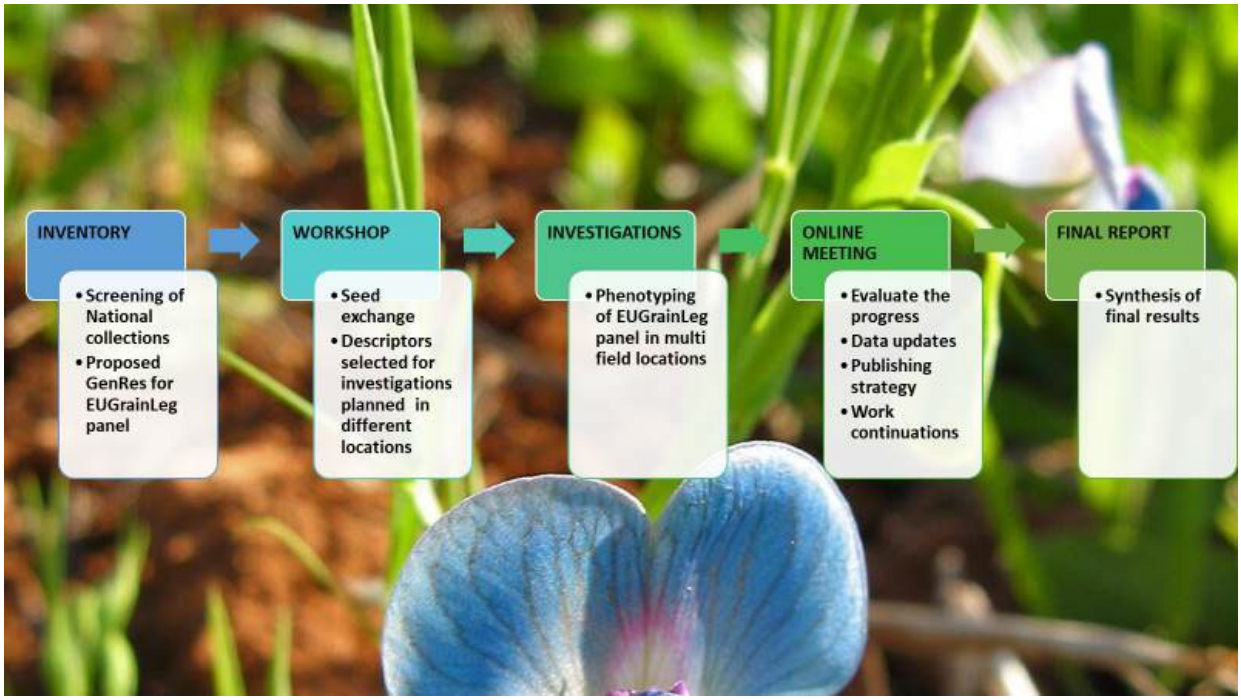

Figure 1. The work plan of EUGrainLeg Activity

RESULTS

The diversity of *Lathyrus* spp. was reflected in presentations offered by partners during the workshop. Related to **Action (1),** inventory and collection of *Lathyrus* genetic material from each of the participating countries' national collections, the partners presented their countries' status as follows: Romania (three species conserved by the National Genebank: *Lathyrus latifolius, Lathyrus sylvestris, Lathyrus sativus)*, Greece (31 species, particular interest in *Lathyrus ochrus, Lathyrus clymenum and Lathyrus sativus),* Bulgaria (around 20 species of *Lathyrus*, the most common species are *L. sylvestris* L., *L. sphaericus* Retz, *L. laxiflorus*, *L. saxatilis* (Vent.) Vis, L. *niger* (L.) Bernh), Bosnia and Herzegovina, Slovenia and Serbia (*L. sativus),* Portugal (21 species present in Portugal's mainland and Madeira, among which the following have accessions conserved in the genebank: *Lathyrus annuus* L. (8); *Lathyrus articulatus L. (8), Lathyrus cicera* L. (13), *Lathyrus clymenum L. (19), Lathyrus hirsutus* L. *(1); Lathyrus latifolius* L. *(1); Lathyrus ochrus L. (17); Lathyrus odoratus L. (7); Lathyrus sativus* L. (111); *Lathyrus sylvestris* L. (6), *Lathyrus tingitanus* L. (9). Thirty-two accessions were not classified until the species rank.

During the presentations, the potential of grass pea for sustainable food production was highlighted, especially because of its limited water requirement and drought tolerance. As the main limitation to its genetic improvement and large-scale cultivation, partners pointed out the low productivity and constraints due to the presence of a neurotoxin (ODAP) responsible for the neurotoxic disorder called lathyrism. Grass pea seeds are used as human food while both seeds and the aerial biomass are used for forage or as an ornamental plant (Polignano *et al.*, 2005; Shwan, 2014; Gixhari *et al.*, 2016).

Discussions were undertaken to develop strategies to allow the identification of genotypes with enhanced yield potential and reduced ODAP content. Briefly, as background for future research investigations: breeding studies exploiting the flower and seed-coat colour correlation with ODAP seed content were not successful (Campbell, 1997). Following many years of breeding, a low ODAP variety (Pusa-24) was released in India by IARI (Indian Agricultural Research Institute). Afterwards, many low ODAP accessions were bred such as Quila-blanco (Chile), Line 8612 (Bangladesh), (Campbell, 1997) and BioL-212, BioR-202, BioR-231, BioI-222, BioL-208, LS 157-12 (Dixit et al., 2016).
In summary, through **Action 1,** partners evaluated the status and the volume of *Lathyrus* collections in the partners' countries. According to this profile and analyzing the availabilities of seeds for exchange to set up feasible experiments in different locations, it was decided the common focus on *Lathyrus sativus.*

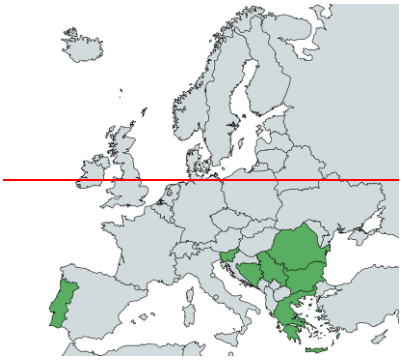

Figure 2. Multi-field locations for investigations planned in EUGrainLeg Activity

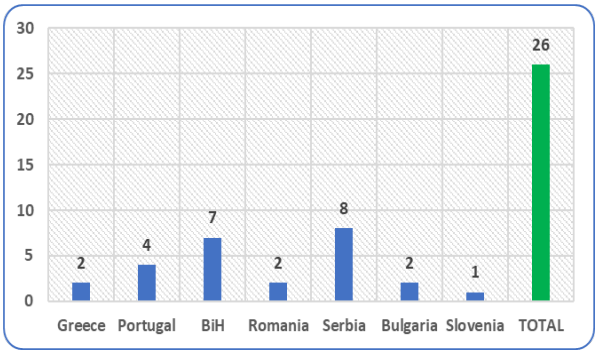

Figure 3. *Lathyrus sativus* panel for EUGrainLeg Activity (number of accessions provided for characterization)

Related to **Action (2),** the establishment of a *Lathyrus sativus* working collection, and exchanges of materials and knowledge were carried out among partners. Partners commonly agreed on providing a minimum number of seeds from their available accessions to be multiplied and phenotypically investigated in different locations of partners. Some materials were exchanged during the workshop, and others via mail after the workshop. The input of each partner, related to the provided number of accessions is represented in Figure 3. The working collection of the EuGrainLeg Activity consisted of 26 accessions. The *Lathyrus* collection, established for investigation, is displayed in Table 1, Annex 1. Each partner investigated up to 26 accessions, a lower number in some cases due to delays in the exchange of materials or failure of experiments, due to different reasons, such as low germination, poor plant development and issues in exchanges via mail.
Regarding **Action (3),** investigations towards the establishment of a high-quality *Lathyrus* data according to the principles of AEGIS; evaluation and characterization of established *Lathyrus sativus* core collection in different environmental conditions upon a common set of traits (phenological, growth habit, pod and seed traits, seed productivity, etc.). The list of traits and details for harmonized data collection are presented in Table 2, Annex 2. Supplementary data were collected by some partners.

EUGrainLeg in Bulgaria. The exchanged accessions were evaluated over the course of two years (2019 and 2020). The exchanged seeds during the workshop in Bucharest (in 2019, 10-11 April) were sown at the first opportunity in the experimental field of IPGR, Sadovo. The seeds from Portugal, Bosnia and Herzegovina and Greece, sent by mail, were sown immediately after delivery (07.05.2019; 28.05.2019; 07.05.2019). Due to the differences in the time of seed's arrival in 2019, some of the accessions were sown not in the most appropriate period for this crop, but in 2020 all accessions were sown properly at the beginning of March.

During vegetation, the plots were weeded and dug by hand. Pest control was performed by two to three treatments mainly against *Bruchus* sp. but also towards some other pests, depending on the degree of infestation.

During the two-year trials, the accessions were evaluated and characterized following the IPGRI '*Lathyrus* spp. Descriptors' - Priority 1 (mandatory), Priority 2 (optional). The accessions were evaluated by all the traits included in Priority 2, except 'brilliance of seed' due to the lack of an assessment scale. Also, they were additionally evaluated for biological yield, harvest index and seed size. During both years, the visual assessments for common diseases and pests were negative except for *Bruchus* sp. in 2019 and *Fusarium* wilt in 2020, causing the death of one accession from Bosnia and Herzegovina (GB01001). During 2019, one genotype with Bulgarian (BGR43334) and one with Romanian (SVGB19385) origin had the highest yield of seeds per plant (20.20g and 20.00g, respectively). These accessions were also characterized by high plants, a big number of primary branches and a high betting of the first pod. During the second year, two accessions from Bosnia and Herzegovina (GB01003 and GB00999) had the highest seed yields per plant (20.00g and 15.20g, respectively). During both years of the study, two other genotypes from Bosnia and Herzegovina (GB01000 and GB01002) had the smallest number of days to maturity (98 days and 103 days, respectively). In 2019 and 2020 one accession from Serbia (KL 5) had the biggest mass of 100 seeds (37.30g and 40.60g, respectively).

The variability of agronomic and biological traits has shown the high genetic *Lathyrus* diversity in evaluated grass pea accessions and increased the possibilities for their use in the breeding activity. Based on phenotyping assessment, several grass pea genotypes were distinguished as initial breeding materials – four from Bosnia and Herzegovina (GB01003, GB00999, GB01000 and GB01002); one from Serbia (KL 5); one from Bulgaria (BGR43334) and one from Romania (SVGB19385).

The investigated accessions are registered in the Bulgarian national database and in EURISCO.

In 2019, the average plant height from all studied accessions was 45.62cm while in 2020 the average height was 57.29cm. The average number of branches was stable and did not vary significantly during both years. The majority of accessions had lanceolate leaflet shape while the remaining genotypes had linear and ovate-lanceolate leaflet shape. Days to flowering, days to first flowering, days to 50% flowering and days to the end of flowering in the year 2019 varied – from 9 to 32 days, from 36 to 46 days, from 40 to 49 days, from 46 to 62 days respectively. During the second year, they varied: from 22 to 36 days; from 53 to 58 days; from 59 to 62 days; from 106 to 111 days. The biggest part of the studied accessions had

days to harvest from 82 to 111 for 2019 and 114 to 122 for 2020 and days to maturity from 89 to 99 for 2019 and from 104 to 109 for 2020. The observed differences between 2019 and 2020 could be explained by the different dates of sowing, being more appropriate in 2020. The colour of the parts of the flower (standard, wing and keel) was determined. The predominant flower's colours were white for standard and keel, and white blue for wings. The average height of the first productive pod in 2019 was 15.58cm while in 2020 it was 22.82cm. Number of pods per plant in 2019 varied from 6.0 (ISOP1176) to 45.3 (KL9) while in 2020 varied from 6.0 (GBO1000) to 52.0 (GBO1003). The largest number of seeds per plant in 2019 was obtained from one Serbian accession (KL9) while in 2020 from one accession from Bosnia and Herzegovina (GBO1003). There were no significant differences in the number of seeds per pod during both years. Most of the studied genotypes had oblong-elliptical pod shape. The average pod length and width of the accessions were 3.92cm and 1.31cm, respectively. All materials had no shattering pods. From all studied accessions, there was a prevalence of rhomboid seed shape, medium seed size, yellow-white, grey-mottled seed coat colour and tubercular seed-coat surface. The largest seed in 2019 was recorded in one accession from Bosnia and Herzegovina (GB000954), and in 2020 in one from Serbia (KL5). There were no significant differences in average seed yield during both years (in 2019, 7.46g; in 2020, 8.85g). During both years, the visual assessments for common diseases and pests were negative except *Bruchus* sp. in 2019 and *Fusarium* wilt in 2020, causing the death of one accession from Bosnia and Herzegovina (GB01001).
An additional activity was the shape investigation: the predominant shape for all accessions was a rhomboid shape. The average seed length and width were 0.87cm and 0.87cm, respectively.
Considering that biological yield and harvest index of grain legumes are important agronomic traits for successful breeding, they were included in the evaluation process. During 2020, the harvest index ranged from 127% (one accession from Serbia, KL5) to 416% (Bulgarian variety Strandja) and biological yield ranged from 7.30g (one accession from Bosnia and Herzegovina, GB01000) to 45.60g (one accession from Bosnia and Herzegovina, GBO1003).
Following the inventory of *Lathyrus* spp. collection at the genebank in IPGR, ten local accessions of grass pea (nine accessions of *Lathyrus sativus* and one of *Lathyrus cicera*) were taken from long-term storage for reproduction and inclusion in AEGIS. *L. cicera* did not germinate. During 2019-2020, these nine *Lathyrus sativus* accessions of Bulgarian origin were reproduced at the experimental field of IPGR, Sadovo. The seeds from these accessions are ready to be included in AEGIS.

EUGrainLeg in Bosnia and Herzegovina: The 19 accessions of grass pea were sown and cultivated in experimental fields at the Institute of Genetic Resources, University of Banja Luka (163m a.s.l.; 44.775°N 17.214°E), Banja Luka, BiH. The field trial was performed during the growing season of 2019, according to the established production techniques.
The trial was carried out under open-field conditions with four seeds/plants per accession. During the growing period, at the relevant plant developmental stages, the individual morpho-agronomic traits for each grass pea accession were evaluated descriptively. Several

Lathyrus spp. descriptors were applied, as designed by the International Plant Genetic Resources Institute (IPGRI, 2000), and agreed by the EUGrainLeg consortium. The grass pea panel was evaluated using a total of 15 quantitative and qualitative morpho-agronomic descriptors for *Lathyrus* spp. that were related to their vegetative growth, inflorescence, stem, branch, leaf, plant, pod and seed data.
All grass pea accessions showed a certain level of morpho-agronomic variability. The most variable traits between accessions were the number of days to 50% flowering, the number of days to maturity and the number of pods per plant. On the other hand, there were no differences observed in the leaflet shape in different accessions. Finally, besides morpho-agronomical characterization, further molecular analyses should be interesting to be performed to have a complete evaluation of grass pea accessions. The complete results are part of Annex 4.

EUGrainLeg in Greece: Greece has a very variable *L. sativus* germplasm. In this context, three Greek accessions along with other 17 accessions from Mediterranean and eastern European countries were characterized at Athens, AUA.
At both growth stages, GR1 and GR2 had the lowest average values for plant height. On the other hand, the highest average was noticed in the accessions SRBKL2 and SRBKL5 for both growth stage measurements, as they reach the 1m height. There were no statistical differences between the accessions provided by Serbia, Portugal, Bulgaria and Romania. The only important statistical differences are between groups a and c. Group a is consisting of the accessions from Greece and Cyprus (GR1, GR2 and GR 3) and group c of the accessions from Serbia (SRBKL2, SRBKL4, SRBKL8, SRBKL9 and SRBKL10), Portugal (ISOP1176, ISOP 1189 and ISOP1190), Bulgaria (BGK40415 and BGK43334) and Romania (SVGB19385 and SVGB20803). The number of primary branches ranged from 4.93 to 11.87 among the *Lathyrus sativus* accessions tested, with SVKSVN 06-66 and ISOP 1176 presenting the highest number of primary branches per plant.
The results indicate that accessions SRBKL2 and SRBKL4 have erect growth habit, the accessions BGK 43334 and GR1 have the most prostrate growth habit, while most of the other accessions are between spreading and semi-erect growth habit. Only one of the accessions tested (BGK 40415) presented ovate-lanceolate leaflet shape, while most of the accessions presented 100% or less linear leaflet shape. In this experiment, two out of the three tested *Lathyrus sativus* landraces of Greek origin, presented 100% linear leaflet shape, while only one of the Greek accessions presented 86.67 and 13.33% linear and lanceolate leaflet shape. Regarding leaf glossiness, 100% of all plants presented glossy leaves and bract size larger than leaf length.
The genetic material from the same origin presented great variability regarding the time interval from sowing to flowering, with accessions from Serbia presenting both the earliest and latest accessions. Greek accessions presented mid to late flowering phenotypes. Flower colour also presented more than one phenotype in 8 out of the 20 tested accessions and was not related to the geographic origin mentioned in literature. Only accessions with 100% white-coloured flowers were one accession from south-eastern Europe (Romania) and one from the Mediterranean region (Slovenia). Greek, Portuguese and Cypriot accessions presented white with blue ribbing and some of the Greek and Portuguese accessions only

blue-coloured flowers. These differences according to Campbell (1997), could be explained by the out-crossing rate of *L. sativus* mentioned by Rahman *et al.*, (1995) reaching up to 28%.

Regarding forage yield traits measured, Greek accessions were less competitive than the accessions that originated from eastern Europe and more northern countries (Slovenia, Bulgaria and Serbia). Pod morphological and agronomical traits were measured at ripening. Almost all the accessions presented pod dehiscence, while only the Serbian accessions SRBKL4 and SRBKL7, and the Romanian SVGB 19385 did not present any dehiscent pod. The lowest mean number of pods per plant (PPPL) was observed in the Greek accession GR2 (32.14) while the highest was in the Portuguese ISOP 1189 (77.90).

Regarding pod width and pod length, accessions in the present study were found with pod width ranging from 0.94 to 1.61cm and pod length from 3.13 to 4.66cm.

More than 70% of the plants regardless of the accessions presented grey-white colour (73.37%), 9.05% yellow-white, 5.53% grey, 4.02% grey mottled, 3.02% green mottled, 2.51% brown, 2.51% yellow-green.

The seed shape of the 20 *L. sativus* accessions tested was (regardless of the accession) flattened at a frequency of 90.91%, while only 9.09% of the seeds were slightly spherical. Spherical seeds were observed in all accessions except those from Portugal.

Seed colour was yellow with some exceptions (GR 3, ISOP 1177 with green, and others – SRBKL5 and SVKSVN 06-66 – with beige).

Regarding seed number per plant, the highest number was observed in AUALIMNOS2018 Greek accession, followed by the Portuguese accession ISOP1189. However, the Greek accession GR 3 had a 100 seed weight of 9.48g. Differently, the Portuguese accession ISOP1189 presented a high seed number per plant and a mean 100 seed weight (15.34g), producing one of the highest yields per plant (22.97g) among the accessions tested.

Seed number per plant and other seed agronomical traits were not related to the country of origin, while the ones with the highest seed yield were ISOP1189 and SRKBL5,8 followed by the small seeded GR 3. Most of the plants (74.29%) presented elliptical pod shape. All Portuguese accessions presented elliptical pod shape.

EUGrainLeg in Portugal: The accessions of grass pea were sown and phenotyped in an experimental field at the University of Madeira, Funchal. Twelve accessions of pea collection from ISOPlexis genebank were screened and characterized in 2019. The sowing date was the end of January (28.01.2019, the most appropriate sowing time) in the open field. In this assay, partners' accessions were not included, because for a *Lathyrus* collaborative assays all accessions were received too late (after Bucharest's meeting, April 2019). A second assay for grass pea accessions characterization was performed in 2020 – 12 accessions were sowed, including 5 Serbian accessions (KL2, KL4, KL5, KL8, KL10), 1 Bulgarian (BGR43334, BGR40415) and 1 Romanian (SVGB19385). The Portuguese partner provided concluding data of three accessions: ISOP 1176, ISOP 1177 and ISOP 1189 investigated in 2019 and 2020; and four in one-year ISOP 1190 (2019), BGR 43334, BGR 40415 and ISOP 1190 in 2020. According to obtained results, the highest seedling vigour was shown by ISOP1189, BGR 43334 and Bulgarian BGR40415 accessions. *Lathyrus sativus* accessions

reached maturity between 99 to 175 days, for Portuguese accessions. Among them were spreading (20.0cm height), and semi-erect and erect (80 to 107cm height) accessions ISOP1176 and ISOP1190, and ISOP 1189. The following productivity and yield parameters were shown by ISOP1176 (107 seed per plant; 22.0g per 100 seeds), ISOP1177 (47 seed per plant; 19.0g per 100 seeds), ISOP1189 (30 seed per plant; 19.0g per 100 seeds) and ISOP1190 (70 seed per plant; 22.0g per 100 seeds). The susceptibility to powdery mildew (*Erysiphe polygoni* f.sp. *pisi*) was detected in all Portuguese accessions. The accessions provided by partners of EUGrainLeg have also been tested, but the majority showed low viability and vigour maybe because the assays were realized in open field and the accessions were not adapted to local agroecological conditions. The results are presented in Annex 4.

EUGrainLeg in Slovenia: 25 accessions were investigated, with only one (GR) having no germination. All accessions were sowed on 07.06.2019. The first flowering appearance was registered from 27 to 39 days, the medium interval being 29,44 ± 2,52 days. The maturity was reached in 86 to 94 days from sowing in a medium interval of 87,76 ± 1,94 days. Seed yield per plant varied from 6,68 to 47,72g, average 15,08 ± 7,81g. GB01003 from Bosnia and Herzegovina was the most productive accession. Similar findings on this accession related to yield per plant were observed also in Bulgaria. The high value of yield per plant was correlated with the number of pods per plant (94 pods).

EUGrainLeg in Serbia: 23 accessions from partner countries were characterized: one accession from Bosnia and Herzegovina and one from Greece did not emerge. One accession from Greece emerged, but only few plants were available and developed poorly and were thus not evaluated. During morphological characterization, the Serbian partner assessed 25 traits. Plant height had an average value of 38,36 ± 10,03cm and varied from 19,20 to 52,50cm. Plants developed 2 to 4,8 primary branches per plant, with an average value of 3,39 ± 0,72cm. Seed yield per plant was inferior compared to other experimental locations. Weight of 100 seeds varied from 12,00g to 34,20g, average 22,85 ± 5,69. Detailed results are presented in Annex 4. The same experiment was repeated during the following growing season (2020). Exchanged accessions are maintained at the IFVCNS genetic collection of *Lathyrus* sp. Ten *Lathyrus sativus* accessions (SRBLAT003, SRBLAT006, SRBLAT002, SRBLAT009, SRBLAT001, SRBLAT007, SRBLAT008, SRBLAT004, SRBLAT005, and SRB AT010) were registered in the EURISCO database.

EUGrainLeg in Romania: A total number of 20 traits were assessed. During the first experimental year (2019), 18 accessions were characterized by VRDS, and only one accession (KL1) from Serbia did not emerge. Accessions from Bosnia and Herzegovina were not subject to investigation in Romania, due to shipment issues. All remaining accessions were successfully reproduced in 2019. In 2020, the second experimental year, 16 accessions were investigated. During 2019 and 2020, the accessions were evaluated and characterized following the '*Lathyrus* spp. descriptors' - Priority 1 (mandatory), Priority 2 (optional); as commonly agreed. Collected data are presented in an MS Excel file, presented as Annex 4 of this report.

In both years, the experiments were established using seedlings produced in alveolus, pictures provided in Annex 3. According to the climatic condition of the region, planting was done in May, in the first decade in 2019 and in the second decade in 2020.
In 2019, two accessions developed poorly vigorous seedlings, four accessions had intermediate vigour, and the rest of the 12 accessions were assessed as developing vigorous seedlings. Plant height (cm) varied from 32,6 to 55,6cm and registered a medium value of 42,01 ± 7,43cm. The medium number of primary branches was 3,94 ± 1,09 with variation from 2,5 to 6,5 primary branches per plant. Plants of investigated accessions requested 35 to 45 days for flower development and a medium interval of 37,61 ± 1,42 days to first flower appearance. The maturity was reached in 83,83 ± 3,76 days, the earliest accessions needed 77 days to reach maturity and the latest accessions 90 days. The medium weight of 100 seeds was 20,37 ± 5,87 and varied from 11,88 to 38,24g. Data related investigated traits and accessions are displayed in Annex 4. The exchanged accessions are conserved at VRDS Bacau and their inclusion in the Romanian national database and EURISCO are ongoing.

CONCLUSION

The Activity development was planned mainly in relation to ECPGR Objectives 1 and 2. The tasks were focused on the characterization and introduction of *Lathyrus* accessions in the European collections.

1. **Visibility and accessibility of PGR material** for potential users via scientific publications, EURISCO and final Report.
   EuGrainLeg concept and results were disseminated as following:

- SINKOVIČ, Lovro, PIPAN, Barbara, ANTIĆ, Marina, TODOROVIĆ, Vida, RAŠETA, Sonja, MEGLIČ, Vladimir. Morpho-agronomic diversity of Lathyrus sativus L. genetic resources from Slovenia and Bosnia and Herzegovina. *Agroznanje*. 2020, vol. 21, no. 2, str. 59-68, ilustr. ISSN 1512-6412. https://doisrpska.nub.rs/index.php/agroznanje/article/view/7105/6908, DOI: 10.7251/AGREN2002059S. [COBISS.SI-ID 60150531]
- Nelly-Lili Fînaru, Alexandru Bute, Ana Maria Nechita, Crina-Andreea Antal, Creola Brezeanu THE GENUS *LATHYRUS*: A NEGLECTED AND UNDERUSED CROP poster presentation at - “Agriculture & Food - 8th International Conference” Burgas, Bulgaria 25.08.2020 – 30.08.2020.
- SINKOVIČ, Lovro, PIPAN, Barbara, MEGLIČ, Vladimir, PETROVA, Sofia, ANTIĆ, Marina, BEBELI, Penelope J., PINHEIRO DE CARVALHO, Miguel A.A., SAVIĆ, Aleksandra, VASIĆ, Mirjana, CREOLA, Brezeanu. *Lathyrus* diversity: available resources with relevance to crop improvement - progress reviews and relationship with AEGIS (EUGrainLeg). V: VAŠKO, Željko (ur.). *Book of Abstracts*. International Symposium on Agricultural Sciences "AgroReS 2020". Banja Luka: Faculty of Agriculture: = Poljoprivredni fakultet, 2020. Str. 61-62. ISBN 978-99938-93-63-9. [COBISS.SI-ID 30256899]
- SINKOVIČ, Lovro, PIPAN, Barbara, SAVIĆ, Aleksandra, VASIĆ, Mirjana, MEGLIČ, Vladimir. Genetic diversity of Lathyrus sativus L. collection and characteristics of seeds produced in Slovenia and Serbia. *Agroznanje*. 2021, vol. 22, no. 3, str. 67-75, ilustr. ISSN 1512-6412. https://doisrpska.nub.rs/index.php/agroznanje/article/view/7650/7434, DOI: 10.7251/AGREN2103067S. [COBISS.SI-ID 78699523]
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- SINKOVIČ, Lovro, PIPAN, Barbara, SAVIĆ, Aleksandra, VASIĆ, Mirjana, MEGLIČ, Vladimir. Genetic diversity of Lathyrus sativus L. collection and characteristics of seeds grown in Slovenia and Serbia. V: VAŠKO, Željko (ur.). *Book of abstracts: X International Symposium on Agricultural Sciences "AgroReS 2021": Trebinje, Bosnia and Herzegovina, 27-29, May, 2021*. Electronic ed. Banja Luka: University of Banja Luka, Faculty of Agriculture, 2021. Str. 39. ISBN 978-99938-93-69-1. https://agrores.net/wp-content/uploads/2021/05/AgroReS_2021_Book_of_Abstracts-3.pdf, https://agrores.net/wp-content/uploads/2021/05/P1_09.pdf. [COBISS.SI-ID 64879619]
- NAGL, Nevena, SINKOVIČ, Lovro, VASIĆ, Mirjana, MEGLIČ, Vladimir, SAVIĆ, Aleksandra, NIKOLIĆ, Zorica, PETROVIĆ, Gordana, PIPAN, Barbara, MARJANOVIĆ JEROMELA, Ana. Evaluation of Lathyrus sativus L. seed for protein content and trypsin inhibitor activity. V: NJEŽIĆ, Branimir (ur.). *Book of Abstracts: XI International Symposium on Agricultural Sciences "AgroReS 2022": Trebinje, Bosnia and Herzegovina, 26-28, May, 2022*. Electronic ed. Banja Luka: University of Banja Luka, Faculty of Agriculture, 2022. Str. 82. ISBN 978-99938-93-81-3. https://agrores.net/. [COBISS.SI-ID 109560067]

2. EUGrainLeg Activity ensured the **characterization and introduction of *Lathyrus* accessions in the European collections**. In relation to expected results and the impact:

- EUGrainLeg provided phenotypic data, improved quality and increased quantity of data and made them available via the EURISCO database; Morphological and agronomical data on selected set of European *Lathyrus sativum* accessions are available according to Outcome 1, output 1.5., act.1.5.3. Conformant to Outcome 1, output 1.2., activity 1.2.1. C&E data have been partially uploaded to EURISCO, but partners are planning to do it. For example, the Romanian partner sent the data to the Romanian Genebank to be uploaded in EURISCO. The Bulgarian partner uploaded 9 accessions in EURISCO, labelled also as part of AEGIS. Acquired information will be useful for end-users (e.g. breeders) Outcome 1, output 1.2., act.1.2.1, Outcome 1, output 1.5.; act. 1.5.3., Outcome 2, output 2.1.; act. 2.1.2, Outcome 5, output 5.4., act. 5.4.1. via EURISCO and

dissemination listed at subsection 1 of Conclusions. The main traits important for future breeding are: time for flowering, pods per plant, yield per plant.

- Propose *Lathyrus* accessions to AEGIS and provide visibility and accessibility of *Lathyrus* plant genetic material for potential users. Outcome 2, output 2.1., activity 2.1.2. and increasing the number of AEGIS *Lathyrus sp.* accessions Outcome 1, output 1.2., act.1.2.1: following the inventory of *Lathyrus* sp. collection at the genebank in IPGR, ten local accessions of grass pea (nine accessions *Lathyrus sativus* and one *Lathyrus cicera*) were taken from long-term storage for reproduction and inclusion in AEGIS. *L. cicera* did not germinate. During 2019-2020 these nine *Lathyrus sativus* accessions of Bulgarian origin were reproduced at the experimental field of IPGR, Sadovo. The seeds from these accessions are included in AEGIS. In Romania exchanged accessions are conserved at VRDS Bacau. The data were sent to be inserted in the Romanian national database and the EURISCO database, via the Vegetal Genetic Resources Bank "Mihai Cristea" Suceava.

3. **The collaboration between ECPGR members/institutes** was strengthened also based on collaboration in EUGrainLeg. Outcome 2, output 2.1., activity 2.1.2. The Grain Legumes members are collaborating within and beyond ECPGR, in the frame of different ongoing international projects and new submissions.
   - Within ECPGR: two new proposals were submitted in the frame of the Sixth Call under the ECPGR Activity Grant Scheme (Phase X): ForEVA and ExploDiv
   - Ongoing international projects such as BRESOV (Romania and Slovenia) and INCREASE (Romania and Slovenia).
   - New proposals in the frame of H2020: HORIZON-CL6-2022-BIODIV-02 (Romania and Bulgaria).

The objectives of the EUGrainLeg Activity were achieved. An extension to the initially planned period was requested due to different reasons. The main reason was the decision to continue in 2020 the characterization started in 2019, considering the difficulties of the first experimental year, including delays, the cultural calendar of grass pea in different regions and seed availability. Moreover, a supplementary task (not assumed in the Activity plan as it was accepted for funding) was decided by partners during the meeting in Bucharest. This was related to the achievement of a gap analysis, with the support of ICARDA (invited observer at the meeting). Unfortunately, the collection and harmonization of all available data and other unpredicted changes impeded the finalization of this task. Another proposed objective was to publish one additional scientific paper. The data and the work are still under evaluation and decision. Considering these two last aims were not assumed officially by partners, the submission of the final report was decided without them.

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ANNEX 1

Table 1. Contributions of EUGrainLeg partners to the panel of *Lathyrus sativus* accessions used in the EUGrainLeg project.

Name	Origin_GeneBank	Material s code as provided by partners	Accession code during investigation
Lathyrus sativus	**Greece**	GR1	**1**
Lathyrus sativus	**Greece**	GR2	**2**
Lathyrus sativus	**Portugal**	ISOP1189	**3**
Lathyrus sativus	**Portugal**	ISOP1176	**4**
Lathyrus sativus	**Portugal**	ISOP1177	**5**
Lathyrus sativus	**Portugal**	ISOP1190	**6**
Lathyrus sativus	**BiH**	GB00999	**7**
Lathyrus sativus	**BiH**	GB01002	**8**
Lathyrus sativus	**BiH**	GB01003	**9**
Lathyrus sativus	**BiH**	GB01001	**10**
Lathyrus sativus	**BiH**	GB00954	**11**
Lathyrus sativus	**BiH**	GB01000	**12**
Lathyrus sativus	**BiH**	GB01004	**13**
Lathyrus sativus	**Romania**	SVGB19385	**15**
Lathyrus sativus	**Romania**	SVGB20803	**16**
Lathyrus sativus	**Serbia**	KL1	**17**
Lathyrus sativus	**Serbia**	KL2	**18**
Lathyrus sativus	**Serbia**	KL4	**19**
Lathyrus sativus	**Serbia**	KL5	**20**
Lathyrus sativus	**Serbia**	KL7	**21**
Lathyrus sativus	**Serbia**	KL8	**22**
Lathyrus sativus	**Serbia**	KL9	**23**
Lathyrus sativus	**Serbia**	KL10	**24**
Lathyrus sativus	**Bulgaria**	BGR40415	**25**
Lathyrus sativus	**Bulgaria**	BGR43334	**26**
Lathyrus sativus	**Slovenia**	SRGB5486	**27**

ANNEX 2

Table 2. Phenotypic traits investigated in EUGrainLeg Activity (from IPGRI, 2000)

Characteristic/trait		Marks/codes						
7.1 Vegetative characters	**7.1.3 Seedling vigour**	Recorded 20 days after emergence						
		3 Poor	**5 Intermediate**	**7 Vigorous**				
	7.1.6 Plant growth habit	Recorded at the beginning of flowering period , see fig 2						
		1 Prostrate	**2 Spreading**	**3 Semi-erect**	**4 Erect**			
7.2 Stem characters	**7.2.1 Plant height [cm]**	Recorded at physiological maturity measured from ground to the tip of the longest branch						
7.3 Branch characters	**7.3.2 Number of primary branches**	Counted at first pod maturity (only on pod-bearing branches)						
7.5 Leaf characters	**7.5.10 Leaflet shape**	See Fig 2. below						
7.6 Inflorescence characters	**Date of sowing**							
	7.6.1 Days to first flowering [d]	Number of days from sowing to when the first flower opens						
	7.6.2 Days to 50% flowering [d]	Number of days from sowing to stage when 50% of plants have begun to flower in a row						
	7.6.4 Days to maturity [d]	Number of days from sowing to stage when 80% of plants have mature pods						
	7.6.12 Flower colour	Score on fresh, open flowers for score **standard**, **wing** and **keel** colours separately						
		1 White	**2 White blue**	**3 Blue**	**4 Grey**	**5 Light yellow**	**6 Yellow**	
		7 Pink	**8 Orange**	**9 Red**	**10 Violet–blue**	**11 Violet**	**99 Other**	
7.7 Pod characters	**7.6.19 Pod-bearing position [cm]**	/height to the first pod. Recorded as height to the lowest pod (see descriptor 7.2.4)						
	7.7.2 Number of pods per plant	Mean number of pods; recorded from randomly selected plants at physiological maturity						
	7.7.4 Pod shape	**1 Oblong-elliptical**	**2 Medium oblong-elliptical**	**3 Curved**	**4 Beaded**	**5 Broad-linear**	**6 Broad-elliptical**	**99 Other**
	7.7.11 Pod length [cm]	Maximum mean length of randomly selected mature pods; recorded at physiological maturity						
	7.7.12 Pod width [cm]	Maximum mean width of randomly selected mature pods; recorded at physiological maturity						
	7.7.16 Number of seeds per pod	Mean number of seeds counted on randomly selected pods; recorded at physiological maturity						
	7.7.17 Pod dehiscence	Scored one week after maturity						

		0 No shattering	**3 Low shattering**	**5 Medium shattering**			**7 High shattering**	
7.8 Seed characters	**7.8.1 Seed shape**							
		1 Oblate or flattened	**2 Triangular**	**3 Rhomboid**	**4 Square**	**5 Obtriangular**	**6 Spherical**	**99 Other**
	7.8.2 Seed size	**3 Small**	**5 Medium**	**7 Large**				
	7.8.3 Seed coat colour	**1 Greyed–white**	**2 Yellow–white**	**3 Grey**	**4 Brown**	**5 Yellow–green**	**6 Pink**	
		7 Red–purple	**8 Black**	**9 Grey mottled**	**10 Green mottled**	**99 Other**		
	7.8.10 100-seed weight [g]	Weight of 100 randomly selected mature seeds at 8-10% (air-dry) seed moisture content						
	7.8.12 Seed yield per plant [g]	Recorded as mean weight of seeds on five randomly selected plants						
Susceptibility to….(visually assessed)		**Bean aphids (*Aphis craccivora*)**				yes/no		
		Pod borers (*Etiella zinckenella*)				yes/no		
		Bruchids (*Bruchus* spp.)				yes/no		
		Jassids				yes/no		
		Powdery mildew (*Erysiphe polygoni* f.sp. *pisi*)				yes/no		
		Downy mildew (*Peronospora lathyri-palustris*)				yes/no		
		Broomrape (*Orobanche* spp.)				yes/no		

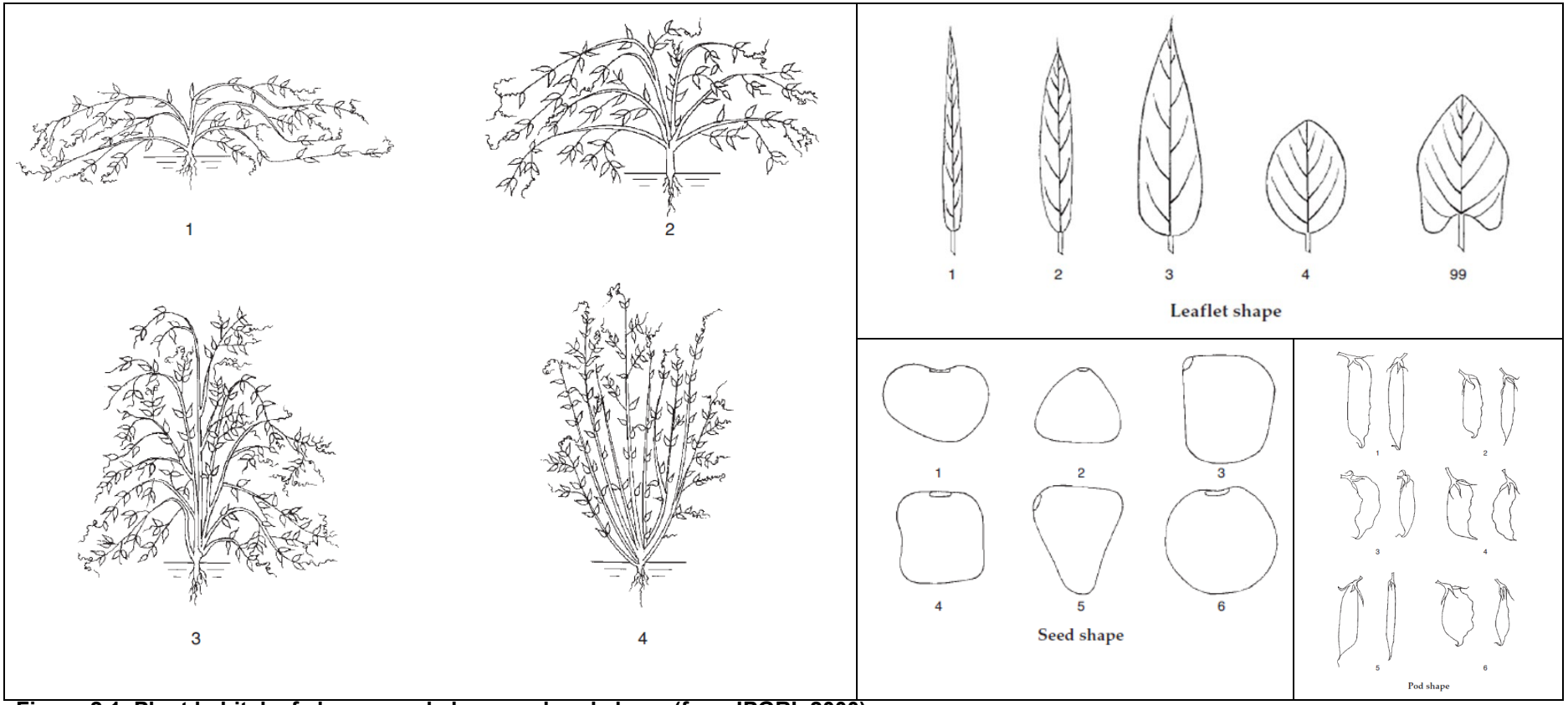
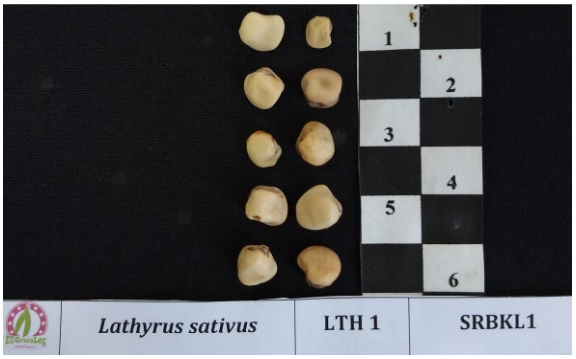


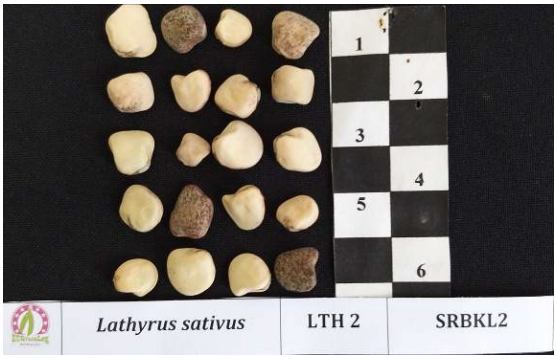
Figure 2.1. Plant habit, leaf shape, seed shape and pod shape (from IPGRI, 2000)

ANNEX 3

3.1 Pictures selection of *Lathyrus sativus* accessions investigated in Romania



(a) ***Lathyrus sativus SRBKL 1***



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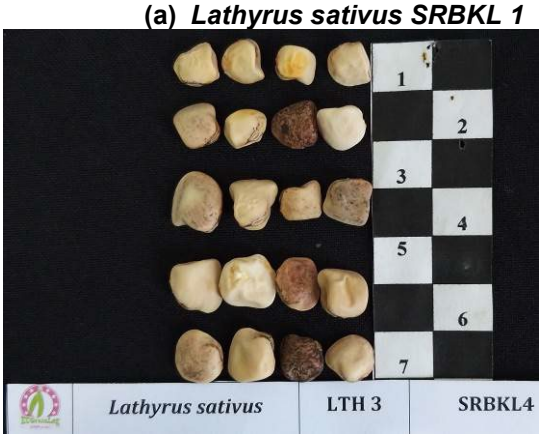
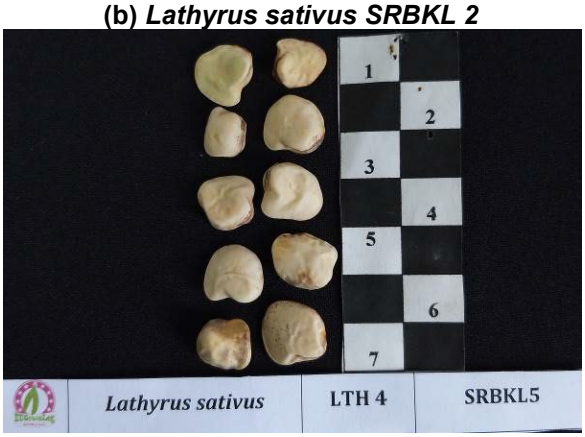
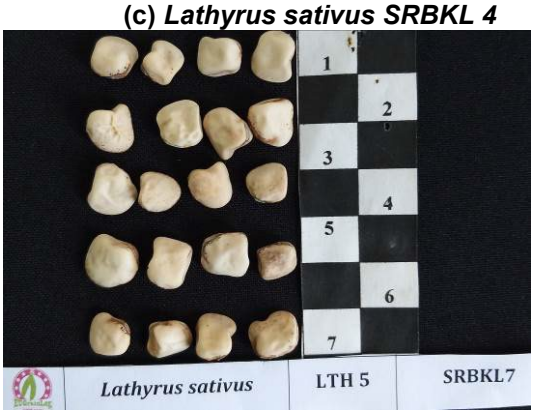


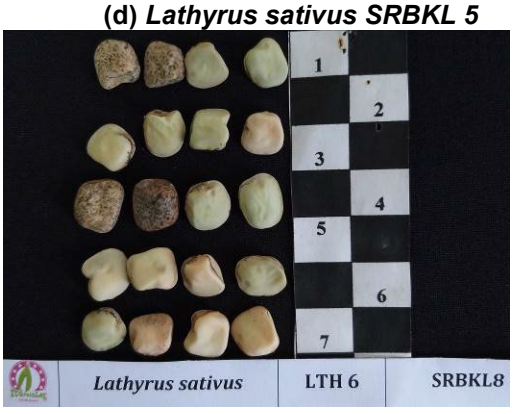
(c) ***Lathyrus sativus SRBKL 4***



(d) ***Lathyrus sativus SRBKL 5***



(e) ***Lathyrus sativus SRBKL 7***



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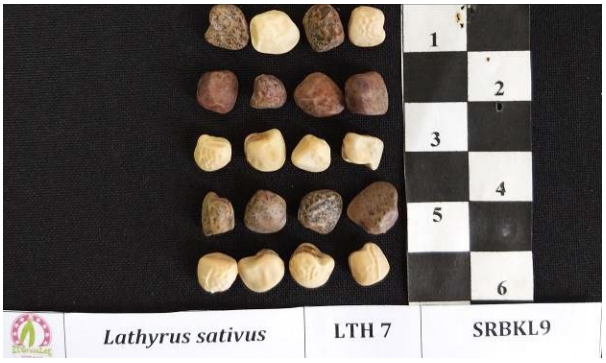
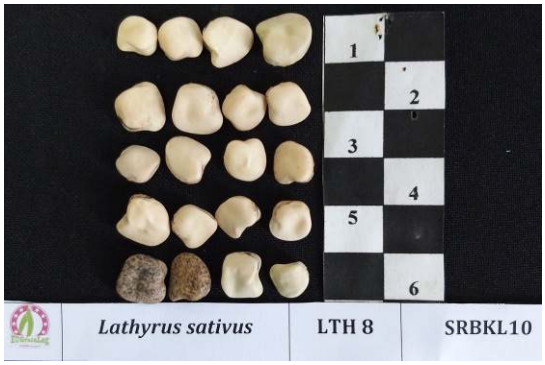


(g) ***Lathyrus sativus SRBKL 9***



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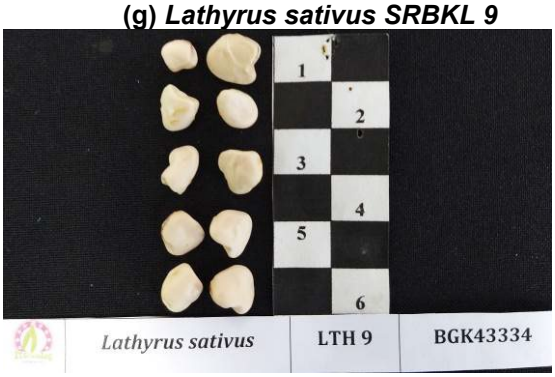
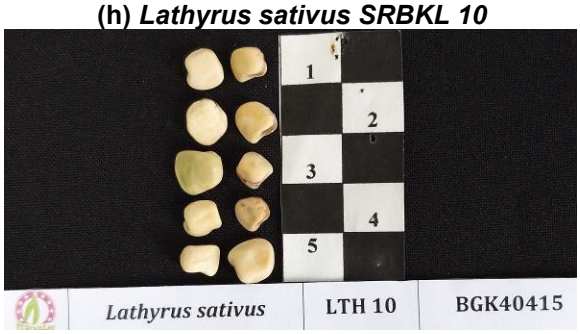
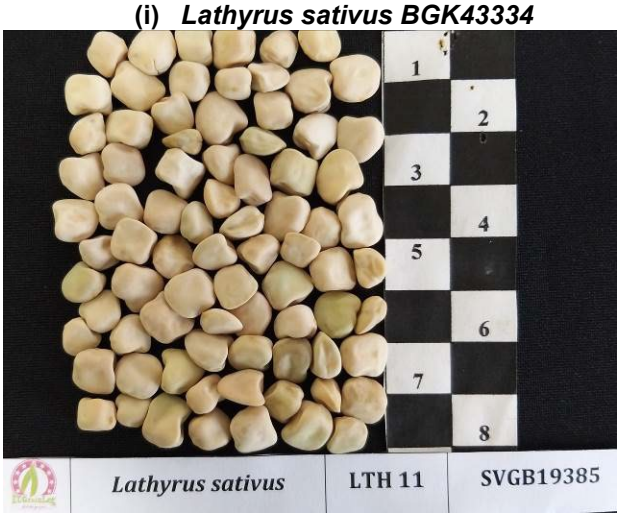


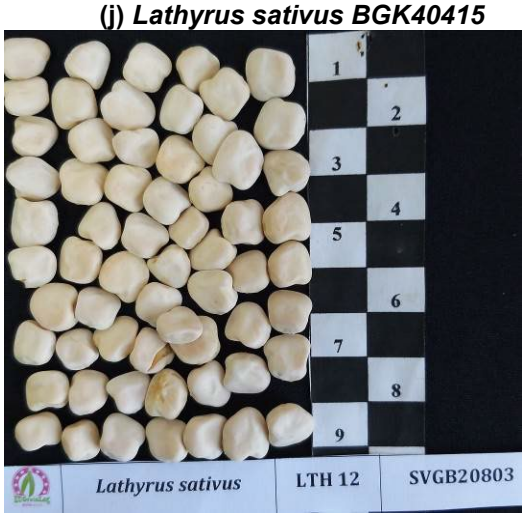
(i) ***Lathyrus sativus BGK43334***



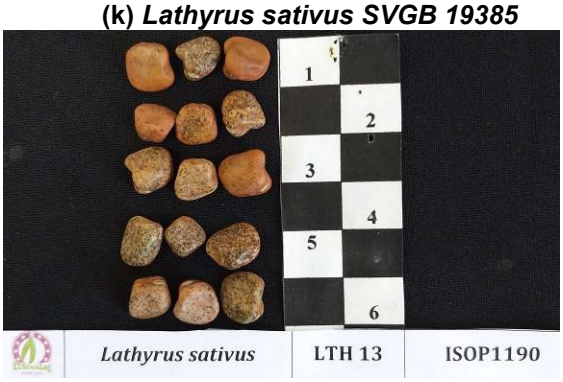
(j) ***Lathyrus sativus BGK40415***



(k) ***Lathyrus sativus SVGB 19385***



(l) ***Lathyrus sativus SVGB 20803***



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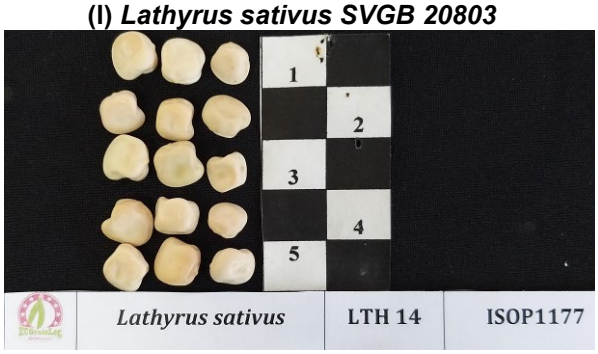
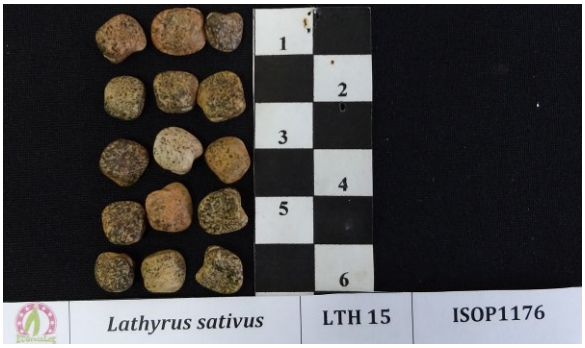


(n) ***Lathyrus sativus ISOP1177***



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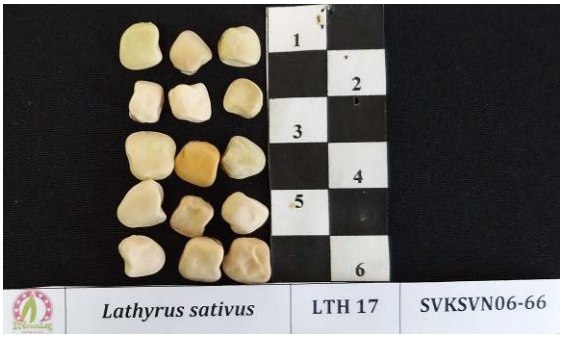
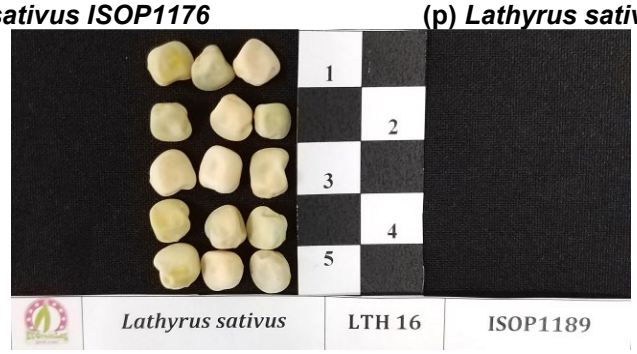
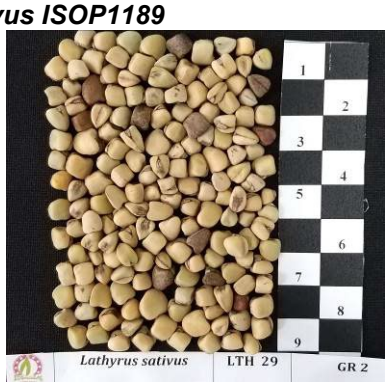


(p) ***Lathyrus sativus SVKSVN06-66***



(q) ***Lathyrus sativus ISOP1189***

(r) ***Lathyrus sativus GR1***



(s) ***Lathyrus sativus GR2***

Figure 3.1.1 a–s Seed of EuGrainLeg *Lathyrus* collection investigated at VRDS Bacau

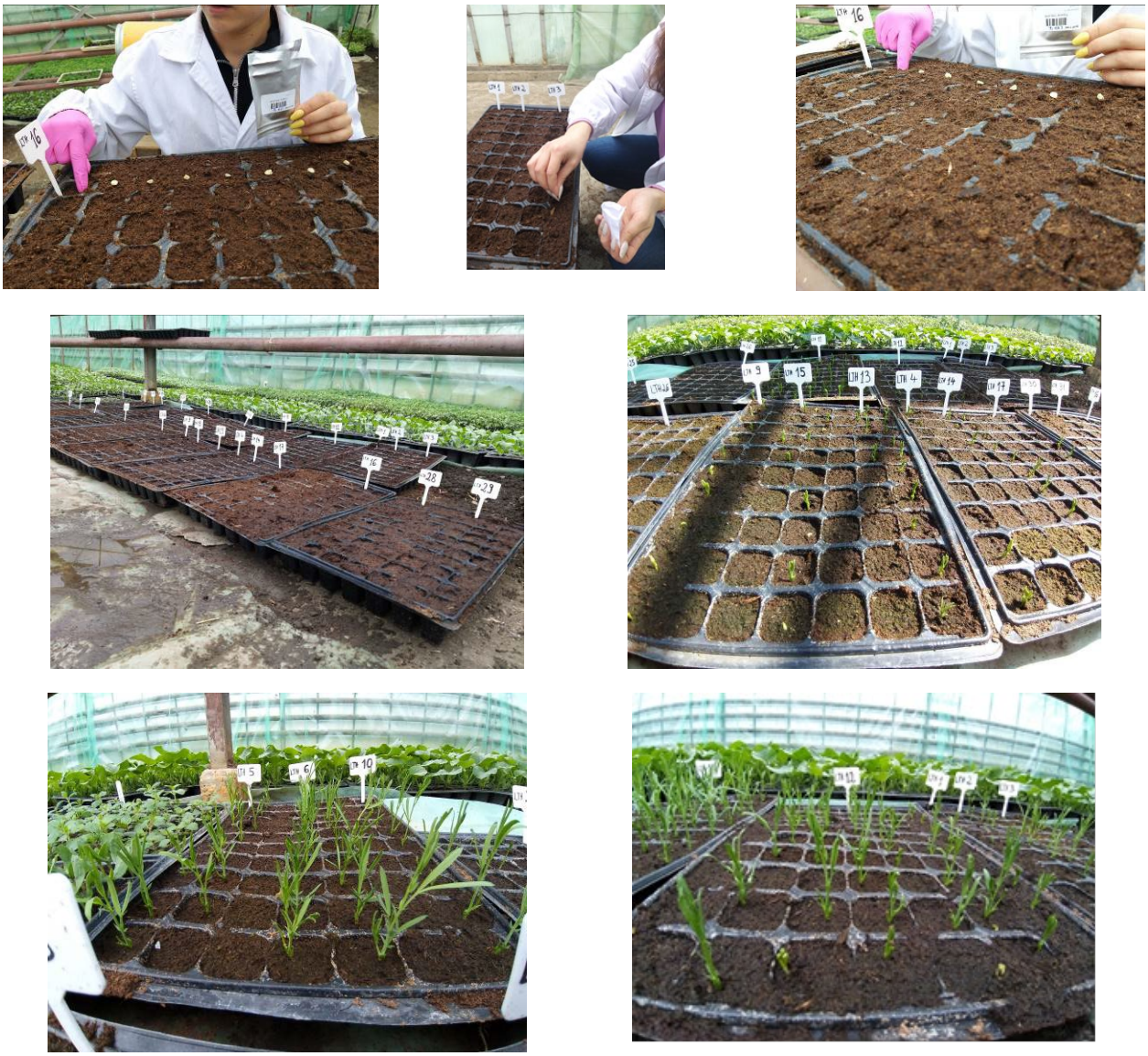


Figure 3.1.2 Sowing, seedlings of *Lathyrus sativus* collection investigated at VRDS Bacau

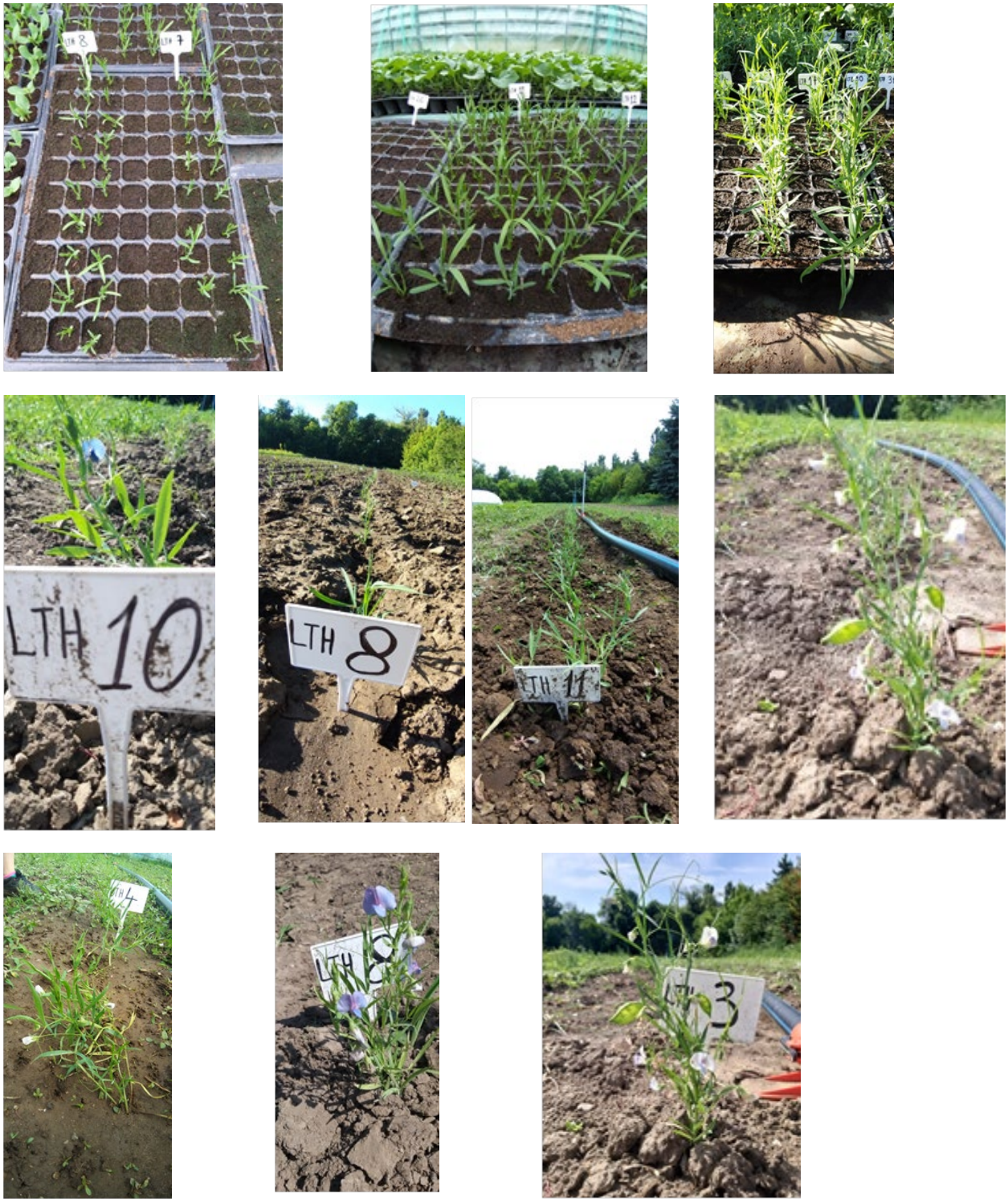


Figure 3.1.3 Seedlings and experimental field of *Lathyrus sativus* at VRDS Bacau

3.2 Pictures selection of *Lathyrus sativus* accessions investigated in Greece

(a) (b)

Figure 3.2.1 (a) The experimental field at the day of plantlet transplantation 2/12/2019; (b) at 15/12/2019

(a) (b)

(c)

Figure 3.2.2 (a): *Lathyrus sativus* field on 13/04/2020; (b) *Lathyrus sativus* field on 22/04/2020; (c) *Lathyrus sativus* field on 28/04/2020.

(a)

(b)

Figure 3.2.3 (a) *Lathyrus sativus* plantlet, 44 DAS (Days after sowing) from the Cypriot accession ARI00290, where one paired leaflets can be distinguished; (b) a two paired leaflet at flowering stage

Figure 3.2.4 Dark green leaflet colour in ISOP 1176 *Lathyrus sativus* accession

Figure 1 *Lathyrus sativus* accession GR 3. Differences at the colour of the flower in the same accession.

Figure 2 *Lathyrus sativus* accession BGK 40415. Differences at the colour of the flower in the same accession.

Figure 3 *Lathyrus sativus* accession ISOP 1190. Differences at the colour of the flower in the same accession.

Figure 4 *Lathyrus sativus* accession SRBKL9. Differences at the colour of the flower in the same accession.

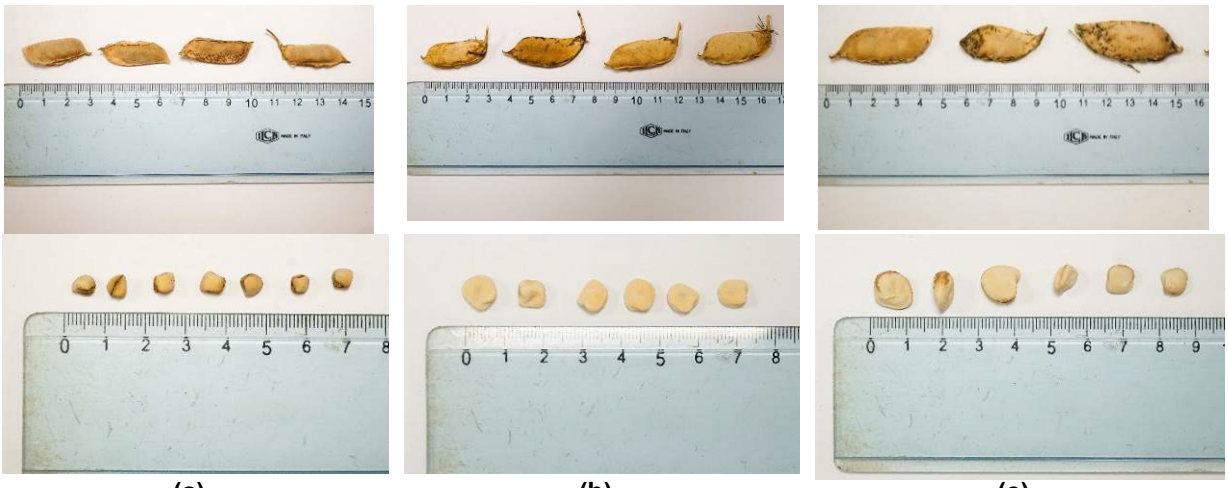
Figure 5 *Lathyrus sativus* accession SVGB 19385. Differences at the colour of the flower in the same accession.

Figure 3.2.10 Accessions (Left) SVGB 19385 rep.II, (Right) ISOP1190 rep.I on 28/04/2020.

(a) **(b)**

Figure 6 *Lathyrus sativus*, ISOP1176 (a) and ISOP1190 (b) accessions aerial biomass at flowering stage.

Figure 3.72 *Lathyrus sativus* SRKBL8 accession, plant aerial biomass at ripening.



(a) (b) (c)

Figure 3.2.13 *Lathyrus sativus* pods and seeds morphology (a) ARI00290; (b) GR2; (c) SRBKL5.

3.3 Pictures selection of *Lathyrus sativus* accessions investigated in Bulgaria

Figure 3.3.1 (a-d) Aspects from Bulgarian experimental device

3.4 Pictures selection of *Lathyrus sativus* accessions investigated in Portugal

ISOP1176 accession

ISOP1177 accession

ISOP1189 accession

Figure 3.4.1 Pictures selection of *Lathyrus sativus* accessions investigated in Madeira, Portugal

ANNEX 4

Phenological investigation (see MS Excel file)