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IMPROVING AND DIVERSIFYING THE CHICKPEA GERMPLASM COLLECTION FOR SUSTAINABLE USE IN BREEDING AND AGRI FOOD CHAIN

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Abstract

Food legumes are crucial for key agriculture-related challenges, such as agrobiodiversity conservation, sustainable agriculture, food security and human health. Comparing to other grain legumes species, chickpea is considered one of the most nutritious for human consumption, having no antinutritional factors except the raffinose-type oligosaccharides, but proven health benefits. One of the major objectives of breeding programs is development of high-yielding, early-maturing cultivars suitable to short cropping season. The current study was developed in frame of national project in ADER program 2019 – 2022 and had as aim the improvement and diversification of chickpea germplasm collection to ensure the optimal use of genetic resources, as a precondition to increase the crop sustainability. The narrow genetic base of cultivated chickpea is one of the major obstacles to sustaining and improving its productivity and renders the crop vulnerable to new biotic and abiotic stresses. In-situ conservation is based on empirical principles and on-farm conservation activities are marginally developed. Seed collections are assembled and are maintained on accession basis, where each accession usually comprises a mixture (population) with an unknown composition of genotypes. This study monitored neutral and functional diversity both at the genetic and phenotypic levels, which means characterizing it, investigating its interactions with the environment in order to define useful functional variation in given environments, establishing a link between variation of phenotypes and —biomarkers to predict potentiality of accessions for specific breeding goals. Plant genetic resources comprising landraces, obsolete varieties and crop wild relatives were investigated. Performance has been achieved by applying new approaches for germplasm characterization and evaluation like development of core sets, mini-core sets, reference sets and trait-specific subsets, etc. Better utilization and conservation of these accessions raises substantial challenges due to the genetic changes that can be occurring during seed multiplication.

***Key words**: biodiversity, Cicer, nutritional profile, quality traits.*

INTRODUCTION

Legume crops as bean, lentil, lupin, mung bean, chickpea, have suffered from the lack of genomic resources for genetic and genomic analysis – they have literally been ‘orphans’ from the genomics revolution (Varshney et al., 2009).

The success of chickpea improvement program largely depends on the wealth of the genetic resources (Ramanappa et al., 2013). Despite its high morphological variability, genetic variation is low (Udupa et al., 1993).

Knowledge about the amount, kind and magnitude of variability in the germplasm and genetic relationships among breeding materials could be an invaluable tool in crop improvement strategies (Joshi and Dhawan, 1966; Kumar and Arora, 1992).

Yadav et al. (2004) observed a negative correlation between yield and seed size under water-limiting environment (Gowda er al., 2011). Developing large-seeded high-yielding kabuli cultivars is an important breeding objective in most chickpea improvement programs. In chickpea, the large-seeded kabuli types produce a lower yield than the small-seeded kabuli cultivars.

Analyzing the available data, we observed there are large gaps between potential and actual yields of chickpea independent of the location of cultivation. New crop rotation and intercropping schemes are tested and encouraged for implementation as key components of the strategy to deal with the biotic stresses that affect chickpea cultivation. In countries with smallholder production, extension work is needed to inform farmers about modern agronomic practices, particularly to enable them to deal with pests and diseases.

The profitability of chickpea is lower than that of cereals like wheat and barley, because of which cultivators with access to irrigation prefer to grow these latter crops (Rawal et al., 2019). In any case chickpea production, needs lower manpower and external inputs than cereals (Sharasia, 2017). Another challenge faced in chickpea production is considerable post-harvest losses because of poor storage infrastructure (Rawal et al., 2019). Average yields of chickpea are nearly 780 kg ha^{-1}, although farmers can harvest more than 2.500 kg ha^{-1}. The crop potential is nearly 5.000 kg ha^{-1} (Sarmah et al., 2012).

According to the FAOSTAT (2017) chickpea is largely cultivated in worldwide in 55 countries. India is listed as the most important producer with 9 million of tons (mt). Among EU countries, are the major European producers, according to the same sources are: Spain, Italy, Bulgaria and Greece. Europe countries specially Spain, Italy, Portugal, Germany, and France import substantial quantities of chickpea from the global market. The analyses of consumption patterns in Europe indicated a rapid increase.

Apart from technological challenges, there are various economic and policy-level issues that need to be dealt with for further expansion of chickpea production.

In the recent years, there has been clear recognition of the role of food legumes in the transition to a plant-based diet, along with the importance of their wider use in the fight against climate change and to promote food security and human health (Bellucci et al., 2021). There are funds allocated in different programs and projects to support and stimulate the conservation for valuable use of legume species. Massive collections are developed by structures that involves phenotypic, genotypic, and agronomic integration to facilitate the access and the use of resources in breeding and along agri food chain.

This work presents the structure of worldwide chickpea collection and investigation results of several traits collected to be integrated and analyzed as potentially useful resources in breeding works. The final aim is to provide new genetic materials, that will benefit growers, seed industry, able to be largely exploited in different cultivation systems under current pressure of climate change. The result of the study will facilitate the exploit of genetic variation of *Cicer arietinum* for enhanced productivity.

MATERIALS AND METHODS

The study is developed in frame of ADER 2020 national research project that deals with the urgent need to provide climate-resilient cultivars technics and methods addressed competitive production systems.

The structure of the paper includes two parts: the first part presents aspects related current situation of chickpea collections as searched in open access catalogs/ data bases of EURISCO and GENESYS, using as filter *Cicer* genus with emphasis on *artiethinum* species.

The second part presents the preliminary results of field investigation aimed to evaluate and to detect the most suitable chickpea resources in terms phenological characterization and yield performance in climatic conditions of Northeast part of Romania, as presented in Figures 1a-1d.

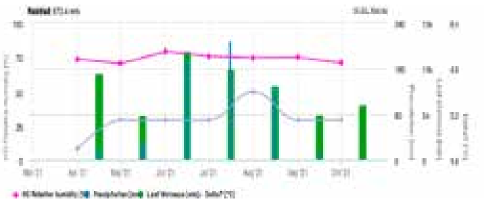

Figure 1a. Relative humidity (%), precipitation (mm), leaf wetness

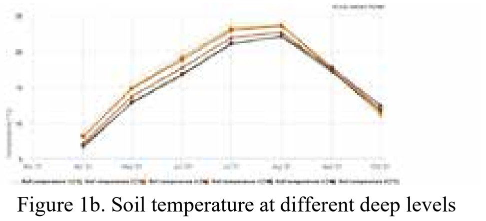

Figure 1b. Soil temperature at different deep levels

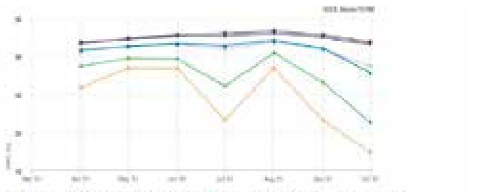

Figure 1c. Soil moisture at different deep levels

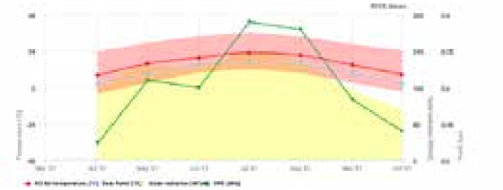

Figure 1d. Air temperature and soil radiation

A collection of eight accessions with different biological status as presented in Figure 2 was established by seed exchanges based on SMTA and collection missions of material from small farms.

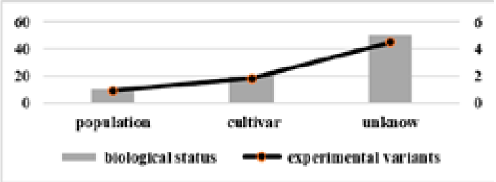

Figure 2. Biological status of investigated material

The heterogeneous biological material studied was collected and maintained as populations During the investigations Single Seed Descent (SSD) lines, meaning seeds derived from a single plant and maintained as pure lines by selfing cycles were developed.

All this material is part reported in EURISCO.

Phenological investigations were conducted by a specific protocol developed using as a reference *Chickpea descriptor* elaborated by Biodiversity International; International Center for Agricultural Research in the Dry Areas (ICARDA); International Crops Research Institute for the Semi-Arid Tropics (ICRISAT); and the Indian Agricultural Research Institute (IARI).

The investigated traits were grouped in three main categories:

- *phenological observations* (sowing date, harvest date, degree of pigmentation and hairiness of the plant, number of days from sowing to early flowering (10%), number of days from sowing to flowering 50%, flowering time 90%, pod ripening)
- *features that impress productivity* (percentage of germinated seeds, number of plants with pods in experimental plot), number of seeds per pod, number of pods per plant, number of seeds per plant, seed mass per plant, appreciation of dehiscence, MMB, resistance and tolerance to pathogen attack)
- *plant architecture* (growth rate of the plant, pod length (mm), plant height (cm), number of leaflets on the leaf, leaf type, canopy height (cm), canopy width (cm), flower color, number of primary basal shoots number of secondary basal shoots number of primary apical shoots).

RESULTS AND DISCUSSIONS

Improving and diversifying the plant germplasm collection for sustainable use in breeding and agri food chain, by creation of Intelligent Collections has been proposed for different legumes species as common bean (Cortinovis et al., 2021), lentil (Guerra-García et al., 2021), and lupin (Kroc et al., 2021) and chickpea (Rocchetti, 2022). The basic principle includes phenotypic characterization, focused on traits considered important for crop adaptation to the different environments (the time to flowering, podding and maturity). Phenology of the crop has an immense influence on productivity and stability. Berger et al. (2006) confirmed the importance of high harvest index (HI) and drought escape in chickpea under natural drought stress through early flowering and maturity. Therefore, most breeding programs aim at developing early-maturing cultivars whose maturity period matches with the available crop duration (Upadhyaya et al., 2010).

According to www.eurisco.ipk-gatersleben.de the European Search Catalogue for Plant Genetic Resources (EURISCO) is a web-based tool that provides information about a significant volume of accessions (more than 2 million accessions of crop plants and their wild relatives), preserved *ex situ.* The net includes about 400 institutes, subject of a network of National Inventories of 43 member countries. The major role of collaborating institutions is supported by a consolidated effort for the preservation of large genetic agrobiological diversity. This online catalog functions based on data automatically received from the National Inventories through country National Focal Points (NFPs). It is in fact a European network of *ex situ* National Inventories (NIs) in purpose to make the European plant genetic resources data available everywhere in the world.

Based on EURSICO National inventory report taxonomy (ipk-gatersleben.de) a total number of 12192 *Cicer arietinum* accessions, and 110 different taxa were detected (Figure 3). Romania has uploaded in EURISCO 127 accessions. The material used for investigations by SCDL Bacau in this work is subject of this collection (Figure 4).

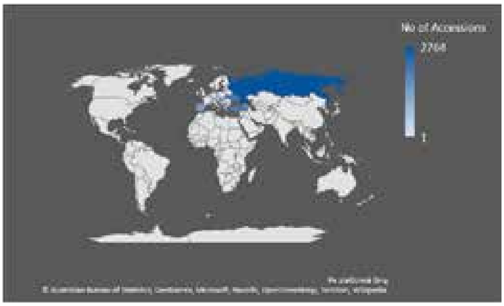

Figure 3. Chickpea accessions availabilities according to EURISCO catalog

Figure 4. Presence of investigated material in this study in EURISCO catalog

Another important tool is represented by Genesys, a database which allows users to explore the world's crop diversity conserved in gene banks through a single website. Genesys brings together some of the world's largest databases of individual gene banks and plant genetic resource networks. These networks often incorporate numerous institutions and many more individual networks – for example, the almost two million accessions provided by ECP/GR through the European Plant Genetic Resources Search Catalogue (EURISCO) come from hundreds of research centers, gene banks and institutions across Europe. Likewise, the CGIAR genebanks are close collaborators and update almost 800.000 passport records every year.

In addition, many individual genebanks, research institutions and smaller networks publish their collection information on the portal to share with the world.

Based on available GENESYS data (https://www.genesys-pgr.org) 66171 *Cicer* accessions are available, 64018 being *Cicer arietinum* accessions.

The largest chickpea collections are maintained at ICARDA and ICRISAT, CGIAR centers with unique accesses estimated at more than 15.000 and 20.000. Collection of wildlife and derived introgression lines is maintained at UC Davis in California. Other worldwide collections of chickpea germplasm Office of National Plant Genetic Resources (NBPGR), India and Gene Banks in Australia, USA, Iran and other countries. The European Chickpea Database was set up in 1996, following the ECPGR meeting in Copenhagen, Denmark, being maintained by the National Institute for Agricultural and Veterinary Research (INIAV). This database includes passport data of cultivated PGR, breeding material and CWR maintained in germplasm collections in European research institutes and gene banks, in line with FAO / IPGRI list according to the Multi-Crop Passport Descriptors. Exchanges and requests for PGR are made based on SMTA directly to the holding institutes. Globally, the need to collect more wildlife has been recognized.

Study of gaps identified in the world collections were:

- the lack of local chickpea varieties in the unexplored areas as Hindu Kush-Himalayas, West and North China, Ethiopia, Uzbekistan, Armenia and Georgia that has been noted for greater diversity;
- the need to collect and investigate CWR which are exploitable sources for tolerance to the pressure of biotic and abiotic factors.

Among the wild species of chickpeas, *Cicer reticulatum* and *Cicer echinospermum*. Chickpea evolved from a unique ancestor, *Cicer reticulatum*.

Diversity is exceedingly low in the modern gene pool, owing to successive human-induced bottlenecks (https://www.pulsesincrease.eu).

Related Cicer species *C. bijugum, C. chorassanicum, C. cuneatum, C. pinnatifidum, C. judaicum, C. yamashitae* and *C. anatolicum*, are annual, apart from *Cicer anatolicum* which is a perennial species (Figure 5).

These wild species have been used to transfer genes for resistance to biotic and abiotic stress. *Cicer bijugum* is reported mainly in western, southern, and southeastern Turkey, northern Iraq, and northeastern Iran, while *C. vistatum* is found in Ethiopia and Pakistan;

- gene transfer from wildlife is not very easy, therefore, studies on characterization, evaluation of various desired characteristics are needed;
- chickpea collections are not fully exploited due to the lack of evaluation data associated with the collected material.

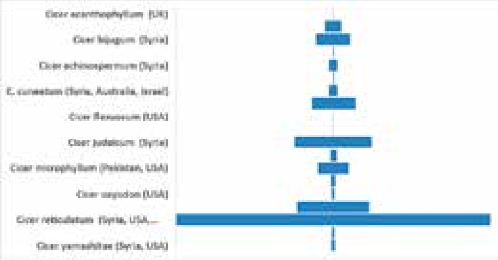

Figure 5. *Cicer* sp. - accessions distribution in different countries

Gene bank collections would become more useful if the collected genetic material will be associated with detailed characterization as well as evaluation data. Chickpeas are suitable for cultivation in extensive agro-climatic conditions that differ depending on the photoperiod, temperature, and precipitation.

A comprehensive screening of chickpea collections revealed a high degree of diversity. Breeders can find and select from global collections genetic resources according to the specifics of their interest. Some web sources able to provide info and plant genetic material are:

- (http://www.genesys-pgr.org),
- (http://www.icrisat.org/),
- (http://www.ars-grin.gov/),
- (http://www.ecpgr.cgiar.org/),
- (http://www.gene.affrc.go.jp/).

The characterization and maintenance of chickpea genetic resources and their exploitation in pre-breeding has major contribution to development of more sustainable agri-food chain.

The collection consisted in eight chickpea resources was investigated in open field conditions for two years in different experimental variants, including use of seedings and direct sowing in conventional versus organic, in soil and in pots. Different periods for experiments development were tested.

Characteristics of investigated materials are summarized in Table 1 and Figure 6.

Table 1. Characteristics of biological material

V	Hair ness	Pig menta tion	Growth habit *	No leaflets	Seeds color **	Testa texture ***
Na1	low	green	5	11	10	3
Na2	low	green	5	13	10	3-5
Na3	low	green	4	13	10	3-5
Na4	low	green	5	11	10	3
Na5	low	violet	4	13	2	7
Na6	low	violet	4	11	1	3
Na7	low	green	2	13	16	3-5
Na8	low	green	2	13	16	3
*	Growth habit	see figure 6				
**	Seed color	beige=10, brown=2, black=1, yellow beige=16				
***	Testa texture	rough=3, smooth=5, tuberculated=7				

Figure 6. Growth habit of investigated material

During the vegetation period, phenological observations and biometric measurements, individual plant harvesting (SSD). Planting was carried out on land modelled on furrows with a crown width of 140 cm, the distance between rows 70 cm, and 20 cm between plants in a row. The comparative chickpea culture included variants displayed in four replicates in random blocks.

The selection and use of high-quality seeds is a first step in inducing a rapid germination process, able to imprint quality and competitiveness of crops. Inoculation, fertilization, and pest control will be of limited value if the seeds sown do not produce a healthy, vigorous seedling.

Sowing pure, high-quality seeds has contributed to: increasing the tolerance of seedlings to disease; promoting the rapid and uniform establishment of the experimental field;

increased tolerance to abiotic stress at the beginning of the season, such as unfavorable temperature and humidity; rapid development of roots; uniform maturity, higher yields and superior seed quality (purity, germination and vigor and disease-transmitted disease levels).

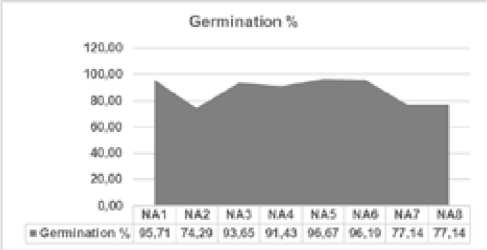

Figure 7. Germination rate at each variant

Three variants recorded values between 74.29 and 77.14%, and five variants germinated over 91%. (Figure 7). Variant Na 5 with a germination of 96.67% was noted as having the highest germination level and the average germination was 87.78% (Table 2).

Table 2. Chickpea germination

	Germination (%)
Min	74,29
Max	96,67
Average	87,78
Standard deviation	9,18
Coeff of variation %	0,10

Regarding the dynamics of the growth and development processes, we found that the first flowers appeared after an interval of 44-68 days from seed emergence.

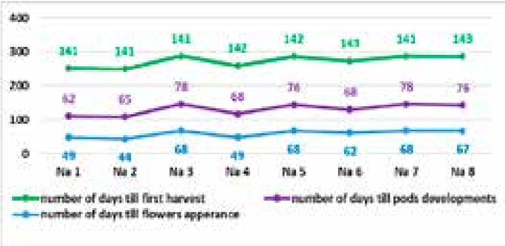

Figure 8. Dynamic of flowering and pods set

The first pods development was observed starting 62 to 68 days after seed emergence. If in the case of the two indicators there were differences between the experimental variants, the first harvests were performed approximately uniformly, after 141 - 143 days from seeds emergence (Figure 8).

The rate of pods maturation (considered at 50% matured by changing color) was between 50 and 65 days after the appearance of the pods. Na 3 recorded the fastest maturation and Na 1 the slowest, in the climatic conditions of 2021.

For a maturation of at least 90%, the experimental variants required an average interval of 78 days from the appearance of the first pods (Figure 9).

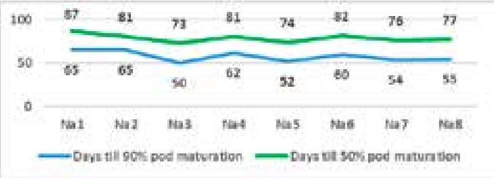

Figure 9. Pod maturation rhythm

We found that Na 3 required 73 days for 90% maturation. Na 1 was noted for its longest interval, 87 days, till 90% maturation. In conclusion, the ripening rate of the pods had a similar dynamic, constant for the two investigated indicators: maturation 50% and minimum 90%.

Table 3. Morphological traits to assess plant architecture

Variant	**Plant height (cm)**	**Canopy height (cm)**	**Canopy width (cm)**	**First pod Insertion (cm)**
Na1	68,00	31,50	82,50	20,50
Na2	61,50	42,50	72,00	21,00
Na3	82,00	48,50	79,00	39,50
Na4	54,50	40,00	69,50	11,00
Na5	84,50	48,00	77,00	34,50
Na6	57,50	34,00	81,00	15,00
Na7	70,00	34,00	61,00	26,00
Na8	74,00	50,00	79,50	26,50
Min	54,50	31,50	61,00	11,00
Max	84,50	50,00	82,50	39,50
Average	69,00	41,06	75,19	24,25
Standard dev	10,81	7,06	7,50	9,72
Coeff of variation %	0,16	0,17	0,10	0,40

The plants height varied between 54.5 cm and 84.5 cm. Variant Na 5 recorded the highest plants, and those with the lowest port were the plants of variant Na 4. This character, cumulated with the first pod insertion height is of particular importance when establishing harvesting methods (Table 3). At the same time, the height of the plants also influences the amount of biomass provided.

In general, all variants had pods with one and very rarely two seeds. Thus, the average number of seeds in the pod varied between 1 and 1.5. Variant Na 2 had the lowest average number of pods per plant, 44.67. Variant Na 3 recorded the highest number of pods per plant (Table 4).

Table 4. Yield components for each variant

Variant	Number of pods per plant	Pod length (mm)	Number of seeds in pod	MMB (g)
Na1	164,33	20,55	1,10	295
Na2	44,67	20,33	1,10	296
Na3	165,33	20,85	1,00	285
Na4	108,33	22,22	1,11	275
Na5	93,25	20,00	1,50	305
Na6	130,67	16,92	1,15	265
Na7	108,75	18,06	1,18	310
Na8	129,58	20,76	1,15	295
Min	**44,67**	**16,92**	**1,00**	**265**
Max	**165,33**	**22,22**	**1,50**	**310**
Average	**118,11**	**19,96**	**1,16**	**290,75**
Standard deviation	**40,91**	**1,76**	**0,16**	**15,41**
Coeff of variation %	**0,35**	**0,09**	**0,14**	**0,05**

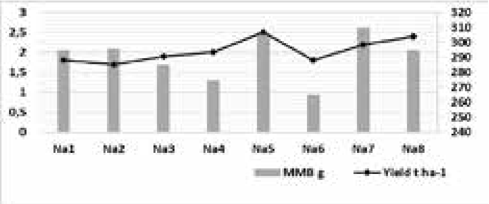

Figure 10. Variation of yield and MMB at investigated accessions

Regarding breeding for a higher yield, chickpeas have an indeterminate character, it is photo-sensitive as well as heat-sensitive, being characterized by a poor management of photosynthesized substances, resulting in low yields. Figure 10 shows the fact that Variants Na 5 and Na 7 were noted for their productivity, and MMB values.

Seed harvesting was done individually from potential plants, identified during the vegetation. Single Seed Descendance is a simple, convenient, inexpensive, and time-saving method, ensuring the possibility of a large number of crosses that can be evaluated by this method, because it requires less space and manpower in each generation. This method preserves considerable variability. The material can be improved quickly. This strategy of developing and using SSD purified accessions has been applied in several projects (e.g., INCREASE, BRESOV); The general conclusion is that SSD lines offer the possibility to associate phenotypes to a unique genotype, thus promoting genetic resources conservation and their use in pre-breeding programs. (Bellucci et al., 2021) (https://www.pulsesincrease.eu/).

CONCLUSIONS

The existing of freely available, national databases is a real support for searching information related the conservation of different species and accessions worldwide.

A special attention is recommended when filter insertion is used in databases, to include synonyms and correct writing of species name.

In terms of chickpea investigation in Northeast part of Romania our preliminary results showed a superior productivity of variant Na 5. Variant Na 3 was highlighted by its earliness.

All variants are retained for the study in competition cultures.

Variants were noted and harvested separately according to performance indices for later use in breeding. Seed harvesting was done individually from potential plants, identified during the vegetation. Single Seed Descendance seems to be a useful tool, simple, convenient, inexpensive and time-saving method, ensuring the possibility of a large number of crosses that can be evaluated by this method. The benefits of the method: it requires less space and manpower in each generation, preserves considerable variability.

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REFERENCES

Bellucci, E., Mario Aguilar, O., Alseekh, S., Bett, K., Brezeanu, C., Cook, D., De la Rosa, L., Delledonne, M., Dostatny, D.F., Ferreira, J.J., Geffroy, V., Ghitarrini, S., Kroc, M., Kumar Agrawal, S., Logozzo, G., Marino, M., Mary-Huard, T., McClean, P., Meglič, V., Messer, T., Muel, F., Nanni, L., Neumann, K., Servalli, F., Străjeru, S., Varshney, R.K., Vasconcelos, M.W., Zaccardelli, M., Zavarzin, A., Bitocchi, E., Frontoni, E., Fernie, A.R., Gioia, T., Graner, A., Guasch, L., Prochnow, L., Oppermann, M., Susek, K., Tenaillon, M., & Papa, R. (2021). The INCREASE project: Intelligent Collections of Food-Legume Genetic Resources for European Agrofood Systems. *The Plant Journal,* 3-13. doi:10.1111/tpj.15472.

Berger, J.D., Ali, M., Basu, P.S., Chaudhary, B.D., Chaturvedi, S.K., Deshmukh, P.S., Dharmaraj, P.S., Dwivedi, S.K., Gangadhar, G.C., Gaur, P.M., Kumar, J., Pannu, R.K., Siddique, K.H.M., Singh, D.N., Singh, D.P., Singh, S.J., Turner, N.,C. Yadava, H.S., S.S. Yadav Genotype by environment studies demonstrate the critical role of phenology in adaptation of chickpea (*Cicer arietinum* L.) to high and low yielding environments of India, Field Crops Research, Volume 98, Issues 2–3, 2006, Pages 230-244, ISSN 0378-4290, https://doi.org/10.1016/j.fcr.2006.02.007 https://www.sciencedirect.com/science/article/pii/S0378429006000694

Cortinovis, G., Oppermann, M., Neumann, K., Graner, A., Gioia, T., Marsella, M., Alseekh, S., Fernie, A.R., Papa, R., Bellucci, E., Bitocchi,, E. Towards the Development, Maintenance, and Standardized Phenotypic Characterization of Single-Seed-Descent Genetic Resources for Common Bean. Curr Protoc. 2021 May;1(5):e133. doi: 10.1002/cpz1.133.

FAO Statistical Databases (FAOSTAT). Rome, Italy. 2017. Available online: https://www.fao.org/faostat.

Gowda, C.L.L, Upadhyaya, H.D., Dronavalli, N., & Singh, S. (2011). Identification of Large-Seeded High-Yielding Stable Kabuli Chickpea Germplasm Lines for Use in Crop Improvement. *Crop Science*, *51*, 198–209.

Guerra-García, A., Gioia, T., von Wettberg, E., Logozzo, G., Papa R., Bitocchi E., Bett K.E., (2021), Intelligent Characterization of Lentil Genetic Resources: Evolutionary History, Genetic Diversity of Germplasm, and the Need for Well-Represented Collections, 2021 - Current Protocols - Wiley Online Library, https://doi.org/10.1002/cpz1.134.

Joshi, A.B., & Dhawan, N.L. (1966). Genetic improvement if yield with special reference to self fertilizing crops. *Indian Journal of Genetics and Plant Breeding*, *26*, 101-113.

Kroc, M., Tomaszewska, M., Czepiel, K., Bitocchi, E., Oppermann, M., Neumann, K., Guasch, L., Bellucci, E., Alseekh, S., Graner, A., Fernie, A.R., Papa, R., Susek K., Towards Development, Maintenance, and Standardized Phenotypic Characterization of Single-Seed-Descent Genetic Resources for Lupins Current Protocols e191, Volume 1, wileyonlinelibrary.com. https:// doi.org/10.1002/cpz1.191

Kumar, L., & Arora, P.P. (1992). Multivariate analysis in chickpea. *Indian J. of Pulses Research*, *5*(1), 1-5.

Ramanappa, T. M., Chandrashekara, K., & Nuthan, D. (2013). Analysis of Variability for Economically Important Traits in Chickpea (*Cicer arietinum* L.).

International Journal of Research in Applied, Natural and Social Sciences, *1*(3), 133-140.

Rawal, V., & Navarro, D. K., eds. (2019). The Global Economy of Pulses. Rome, *FAO*. 21-36.

Rocchetti, L., Gioia, T., Logozzo, G., Brezeanu, C., Guasch Pereira, L., De la Rosa, L., Marzario S., Pieri, A., Fernie, A.R., Alseekh, S., Susek, K., Cook, R.D., Varshney, R.K., Agrawal, K., Hamwieh, A., Bitocchi, E., Papa, R. (2022) Towards the Development, Maintenance and Standardized Phenotypic Characterization of Single-Seed-Descent Genetic Resources for Chickpea, Current Protocols e371, Volume 2 wileyonlinelibrary.com, doi: 10.1002/cpz1.371

Sarmah, B.K., Acharjee, S., & Sharma H.C. (2012). Chickpea: Crop Improvement under Changing Environment Conditions. *Improving Crop Productivity in Sustainable Agriculture*, First Edition. 362-364.

Sharasia, P.L.; Garg, M.R.; Bhanderi, B.M. Pulses and Their by-Products as Animal Feed; Calles, T., Makkar, H.P.S., Eds.; FAO: Rome, Italy, 2017

Udupa, S.M., Sharma A, Sharma, R.P., & Pai, R.A. (1993). Narrow genetic variability in Cicer arietinum as revealed by RFLP analysis. *Journal of Plant Biochemistry and Biotechnology*, *2*, 83–86.

Varshney, R.K., Hiremath, P.J., Lekha, P., Kashiwagi, J., Balaji, J., Deokar, A.A., Vadez, V., Xiao, Y., Srinivasan, R., Gaur, P.M., Siddique, K.H.M., Town, C.D., & Hoisington, D.A. (2009). A comprehensive resource of drought- and salinity-responsive ESTs for gene discovery and marker development in chickpea (*Cicer arietinum* L.). *BMC Genomics*, *10*, 523–541.

Yadav, S.S., Kumar, J., Turner, N.C., Muehlbauer, F.J., Knights, E.J., Redden, B., McNeil, D., & Berger, J. (2004). Enhancing adaptation of large seeded kabuli chickpea to drought prone environments. 116-117. In Fischer, T. et al. (ed.) New directions for a diverse planet: *International Crop Science Congress*, 4th, Brisbane, Australia, 26 Sept.–1 Oct. 2004. Regional Institute Ltd., Gosford, Australia.

Upadhyaya, D.H., Yadav, D., Dronavalli, N., Gowda,C.L.L, Singh, S., (2010) Mini core germplasm collections for infusing genetic diversity in plantbreeding programs Electronic Journal of Plant Breeding, 1(4): 1294-1309 Available from: https://www.researchgate.net/publication/46194405_Mini_core_germplasm_collections_for_infusing_genetic_diversity_in_plant_breeding_programs (accessed Mar 14 2022).

Web sources

- http://www.genesys-pgr.org,
- http://www.icrisat.org/
- http://www.ars-grin.gov/
- http://www.ecpgr.cgiar.org/
- http://www.gene.affrc.go.jp/
- EURISCO: European Search Catalogue for Plant Genetic Resources (ipk-gatersleben.de)
- https://www.pulsesincrease.eu
- https://www.bresov.eu