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Lentil Spanish Germplasm Characterization: Seed Composition and Agronomic Performance Evaluation

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Abstract: Lentil (*Lens culinaris* Medik) is an annual herb that belongs to family Fabaceae, it is one of the most ancient food crops that has been grown in the world, and originated from southwestern Asia as early as 7000 BC. In Spain lentil acreage raised 42,200 ha in 2022, two authonomous regions Castilla-León and Castilla-La Mancha are traditionally the large producers. Lentils have the capacity to fix atmospheric N_2 in symbiosis with rhizobia and, additionally it is considered moderately tolerant to drought which in turn makes this pulse a low water and carbon fingerprint crop. The aim of this study was to assess the symbiotic responsiveness to rhizobia strains of several lentil accessions from the Spanish germplasm bank, to determine the nutritional value and mineral seed composition and, to evaluate the agronomic performance of six selected accessions in two regions under rainfed conditions and different management systems. The protein content of all genotypes was higher than 25%, with two outstanding seeds from accessions: BGE025596 and BGE026702 which account for >30% of protein and <2% of fat. Accessions BGE016362 and, BGE026702 had an exceptional Fe content, > 1 g/100 g of seed flour. The agronomic performance varied depending upon the cropping región.

Keywords: Lentils; Rhizobium; seed nutritional composition; seed mineral composition; phenolic compounds

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1. Introduction

Lentil (*Lens culinaris* Medik) is an annual herb that belongs to family Fabaceae, it is considered a moderately tolerant to drought. Canada is the largest lentil producer in the world with 2 millions tonnes production per year. India comes the second with 1,6 millions yearly production and, USA with 381,380 tonnes is the third largest producer in the world (atlasbig.com/en-gb). Yield of lentils across the ten top world producers ranges from 730 to 1,400 Kg/ha. In Spain lentil acreage raised 42,200 ha in 2022 [1]; two authonomous regions of Central and North Spain (Castilla-León and Castilla-La Mancha) are traditionally the best producers with a 12% of the national acreage dedicated to lentil cropping. As most of the grain legumes lentils in Spain are basically cultivated under rainfed conditions.

Rhizospheric bacteria named rhizobia are able to form mutualistic associations with legume plants, during which they fix atmospheric nitrogen and provide it in a form that is readily available to plants, in exchange for carbon compounds from photosynthesis [2], thereby in most cases, N-fertilizers are not needed or recommeded to complete the crop cycle. These exceptional characteristics of low water and N requeriments make legumes indispensable and key crops for a sustainable and hard forecast scenario in Agriculture practices due to the cllimate change. The selection of specific and highly efficient rhizobia strains compatible with the target legume still being a critical step when new species or plant accessions are intended to be cropped relaying on nitrogen fixation. Additional advantages of legumes are also related with its positive role in intercropping systems with cereals and other crops, as the improvement of soil characteristics, the enrichment on nitrogen and organic matter content.

Most countries face some form of malnutrition, ranging from undernutrition and micronutrient deficiencies to obesity and diet-related diseases. In this context, pulses are important food crops and should be part of a healthy diet because they are recognized as being readily available sources of protein, complex carbohydrates, fibres, vitamins, minerals, and bioactive compounds [3], while being low in fat [4,5]. For nutritionists, pulses are considered healthy and high nutrient-like protein-rich diet, that mainly decrease the risk of stroke and heart diseases. Even though grain legumes were part of many traditional diets, pulse consumption has decreased globally.

Spanish landraces were grouped based on their seed weight instead of their phenology [6], thus following conclusion obtained by [7] about phenology is a major factor in adaptation on macrogeographic scale but at microgeographic level it is of lesser importance. As seed weight is generally a trait of low adaptable value and readily observable [8], it seems that human preferences could have been a relevant factor to determine grouping of Spanish lentil landraces. Some studies [6,9], found that *microsperma* type was more polymorphic than *macrosperma* type. We have worked with lentil germplasm from Plant Genetic Resources Centre (CRF), with the aim to contribute to the conservation of food-legume genetic diversity and its exploitation in food production [10], which in turn will also increase the sustainability of agriculture and the availability of healthier food products. To our best knowledge, this is the first study concerning agronomic performance evaluation, nutritional, mineral and phenolic compounds seed characterization of local landraces or accessions. At the same time, responses of lentil accessions to rhizobia strains have been addressed.

Key words: lentils, *Rhizobium*, plant x rhizobia interactions, seed composition

2. Material and Methods

2.1. Plant material

Seeds of lentil accessions were provided by the Plant Genetic Resources Centre (CRF). The augmentation of seeds was carried out at the Agriculture Experimental Station Albaladejito (IRIAF, Cuenca) during 2021. Accession number code and origin of the lentils used in this work are listed in Table 1.

2.2. Rhizobium strains and culture media

Bacterial strains used in this work are listed in Table 2. *Rhizobium leguminosarum* strains were routinely grown in yeast-mannitol (YM) [11] medium or triptone-yeast extract (TY) medium [12].

2.3. Plant test under controlled conditions

Two plant trials, under greenhouse or plant growth chamber conditions, were carried out on lentils plants growing in 0.5 l Leonard jars [10], filled with a perlite-vermiculite mixture (1/2 v/v) and watered with N-free nutrient solution [13]. Plant tests aimed at determining the symbiotic capacity of *Rhizobium leguminosarum* strains were carried out on lentil accessions surface disinfected and pre-germinated (Tables 3A and 3B). The numbers of native rhizobia in soil samples were estimated by the most probable number (MPN) counting technique using the commercial small seeded variety Pardina as plant host [14].

2.4. Soil characteristics and cllimate of experimental areas

Soil characteristics and estimations of soil rhizobia density (MPN) are shown in Table 4A, climate main conditions of the field experiments areas are presented in Table 4B.

2.5. Field experiments

Two field experiments (FE) were carried out at the Agriculture Research Station IRIAF-Albaladejito (Cuenca, Spain) (40º04´ N, 2º08´ W, 997 masl) and, at the Agriculture Research Station IFAPA-Tomejil (Carmona, Seville, Spain) (37º28´ N, -5º38´ W, 253 masl) during 2021-2022. Experimental fields were laid out in a randomised complete block design with three replicates. Within each block, there were plots of 1.2 x 7m (divided into rows, spaced 0.5 m apart). A space of 0.5 m was allowed between plots and 2.5 m between blocks. A planting density of 300 plants/m^2 for

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monocrop system and, 200 plants/m² for intercropping mix seeds system (160 lentil plants and 40 barley plants) was used. In all inoculated treatments, seeds were inoculated with perlite-based inoculants following [15] and, composed of two strains (GU-2 and HA-2) [16]. Results of grain yields in Tomejil (Seville) and Albaladejito (Cuenca) are shown in Tables 5A and 5B, respectively.

2.6. Proximate and mineral composition of seeds

Dry and raw seeds of lentil accessions were ground and sieved at 1 mm to obtain the corresponding flour. Samples flours were sent to authoritative specialized analyses unit Laboratorio Agroalimentario de Córdoba, (AGAPA) for proximate and mineral composition determination. The constituents referred as *mandatory nutrition declaration* (energy, fat, carbohydrates, sugar, salt, and protein) on REGULATION (EU) No 1169/2011 (art. 30) [17] plus fibre content, ash, and humidity were determined. Mineral components: N, P, K, Ca, Mg, Na, Fe, Mn, Cu and Zn were determined by ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometer). We have analyzed the six lentil accessions that have been evaluated under field conditions. Results of proximate and mineral composition are shown in Tables 6 and 7, respectively.

2.7. Phenolic compounds determination

Methanolic extracts (methanol:water, 70:30) of lentil flours were analysed by UHPLC-HRMS (Ultra High Performance Liquid Chromatography-High Resolution Mass Spectrometry) by the target screening method against more than 90 phenolic compounds at CITIUS (Centre of Research, Technology and Innovation University of Seville, Spain). Results of phenolic compound composition are shown in Table 8.

3. Results and Discussion

In this study we have employed eight accessions of *Lens culinaris* Medik. coming from Central Spain (Castilla-La Mancha region): Guadalajara, Ciudad Real and Albacete provinces, South Spain (Andalusia region): Jaén and Granada provinces, Canary Islands: Lanzarote and Fuerteventura Islands and, one from León province (North Spain), (Table 1). These accessions come from a wide geographic area with very different ecological conditions. To our knowledge this is the first study evaluating the symbiotic response to rhizobia strains and, seed composition of Spanish lentil accessions from Plant Genetic Resources Centre (CRF). Other studies related with the evaluation of agro/morphological traits of one hundred Spanish landraces have been previously published [6]; in that survey authors divided the Spanish lentils in two clear morphological groups based on seed characters: *macrosperma* and *microsperma* types.

3.1. Plant test under controlled conditions

All studied accessions did positively respond to inoculation of several rhizobia strains under controlled conditions (Tables 3A and 3B), albeit with large differences in terms of symbiotic efficiency, thus showing a high plant genotype x rhizobia strain interaction. In general, *microcarpa* type accessions, did show a superior symbiotic performance. From these trials we selected two strains HA-2 and GU-2 to further evaluation under field conditions. A first trial involving inoculation of four different lentil accessions, two *macrosperma* type (from Albacete and Jaén) and, two *microsperma* (from Granada and León) with four especific rhizobia strains (Table 3A) led to a positive response in terms of shoot biomass accumulation in comparison with non-inoculated plants as there was significant differences with uninoculated plants, except for some combinations as ISL37 x Albacete and, GU-2 and ISL55 x Jaén (Table 3A). The nodulation capacity (number of nodules and nodule mass) in most accessions was superior with the strain HA-2, as reflected in its symbiotic efficiency, with some exceptions as combinations ISL37 x Jaén and GU-2 x Granada; although in this Granada accession the symbiotic efficiency was higher in combination with the strain ISL37. On the other hand, lentil accessions of *microsperma* type showed a higher symbiotic efficiency to all inoculant strains in comparison with *macrosperma* types. In addition these two *microsperma* accessions (local

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denomination *verdina*) have dark-green pigmented testa, thus in agreement with other works [18,19], that have demonstrated that colorured seeds varieties of *Phaseolus vulgaris* and *Vigna subterranea* had a superior nodulation and N_2 fixation capacity. Correlations among variables plant biomass and nodule biomass was high and positive for all rhizobia strains ranging r= 0.78-0.90; lower but positive correlation was found among variables plant biomass and number of nodules, which ranged r= 0.33-0.66. From this trial we selected strains HA-2 and GU-2 to extend the evaluation with other accessions and, further evaluation under field conditions.

In a second plant trial, 6 lentil accessions were evaluated -two in common with the first trial- and the selected strains HA-2 and GU-2 were used as inoculants. (Table 3B). All accessions responded positively to inoculation in terms of shoot biomass accumulation in comparison with non-inoculated plants. In general, plants accumulated more biomass when inoculated with the strain GU-2, as reflected by the high symbiotic efficiency. The nodulation capacity (number of nodules and nodule mass) of both strains was similar in each accessions. Correlations among variables plant biomass and nodule biomass was high and positive for both strains (r= 0.75-0.79), nodule number and nodule biomass correlations ranged r= 0.74-0.84; lower but positive was the correlation among variables plant biomass and number of nodules, which ranged r= 0.41-0.50.

3.2. Field experiments

In this study we have employed eight accessions of *Lens culinaris* Medik. coming from Central Spain (Castilla-La Mancha region): Guadalajara, Ciudad Real and Albacete provinces, South Spain (Andalusia region): Jaén and Granada provinces, Canary Islands: Lanzarote and Fuerteventura Islands and, one from León province (North Spain), Table 1. These accessions come from a wide geographic area with very different ecological conditions. To our knowledge this is the first study evaluating the symbiotic response to rhizobia strains and, seed composition of Spanish lentil accessions from Plant Genetic Resources Centre (CRF). Other studies related with the evaluation of agro/morphological traits of one hundred Spanish landraces have been previously published [6]; in that survey authors divided the Spanish lentils in two clear morphological groups based on seed characters: *macrosperma* and *microsperma* types. Field experiments were set up at two locations with different edaphoclimatic conditions (Tables 4A and 4B), which could partially explain the differing grain yield results. Results of the FE at IFAPA-Tomejil, shown that grain yields of the accessions from Canary Islands and those of *verdina* type (from León and Granada) significantly outstand grain yields of two others accessions under conventional monocropped systems (Tabla 5A). Seed inoculation, with a specific composed inoculant of two *R. leguminosarum* strains (GU-2 and HA-2), did not increase seed yields over un-inoculated monocrop treatment. Under intercropping system with barley, the accesions from Fuerteventura Island and Granada both outstand yields of the others. Under intercropping managenent lentil yields were not reduced in most accessions, exceptions were Lanzarote Island and León accessions. Under climate conditions of South Spain experimental area, located at low altitude, less rainfall and high mean year temperature (Table 4B), accessions from the North of Spain provinces (Guadalajara and Ciudad Real), did yield significantly less than the others accessions, in contrast to grain yields in FE-Albaladejito.

Results of the FE at IRIAF-Albaladejito shown that under conventional monocrop conditions grain yields of the accessions from Guadalajara and Granada did significantly outstand the others (average 1.5-4.7 fold) (Table 5B), accessions from Canary Islands did not have a good performance under these climate conditions with low temperature throughout the year and during the crop development in winter season (Table 4B). Two accessions (from Ciudad Real and León) did positively respond to inoculation with specifc rhizobia strains (inoculated-monocrop *vs* monocrop), with grain gains of 61 and 21%, respectively; which resulted unpredictable as the rhizobia size population in this soil (Table 4A) was above the recommended rhizobia density that may preclude the use of inoculants, or to expect a positive response to inoculation [20]. In this study, authors shown that the probability of enhancing yield decreases dramatically with increasing numbers of indigenous rhizobia. The response to inoculation and the competitive success of inoculant rhizobia were inversely related to numbers of indigenous rhizobia. As few as 50 rhizobia g of soil eliminated inoculation response. In general, intercropping management system did not negatively affect grain yield of most accessions, exception made of accessions from Fuerteventura and Granada. In summary, although Seville field area accumulated less rainfalls -along cropping season- than Cuenca (191 mm *vs* 443 mm) of which 107 mm and 70 mm, respectively, fell in march during full pod development period, the clay type soil of Seville experimental area could account for a better water reservoir till harvest, wich in turn could explain the general higher yields.

3.3. Proximate and mineral composition of seeds

Pulses are an excellent source of key nutrients, including carbohydrates (e.g., fiber and starch), vegetable protein, folate, other vitamins, and minerals like potassium and iron with health-promoting benefits. Pulses are a rich source of essential micronutrients, such as iron, potassium, magnesium, zinc, and B. Daily mineral requirements can be satisfied by taking 100–200 g pulses (lentil, cowpea, and chickpea), and daily iron requirements can be met by consuming 100 g of most dietary legumes [3]. The proximate composition of seeds is presented in Table 6. We have determined the constituents referred as *mandatory nutrition declaration* on [17], plus fiber, ashes, and humidity content. The nutritional constituents of lentil accessions were contrasted with values reported in FAO [4], for 21 lentil entries and, all comparisons were made based on mature, whole, dried, and raw seeds data. Energy values of our samples averaged 343 Kcal, thus below but consistent with the reported values of 355-375 Kcal; available carbohydrates, and fat contents showed to be higher (averages of 55.7 and 2.05 g/100g, respectively) in our samples in comparison with reported values. The two *verdina* accessions showed the lowest fat (< 2%) and carbohydrate contents of the analyzed Spanish accessions. Reported values, in FAO food composition guide, of fibre total dietary (ranging 11.7-19 g) are superior to that of our samples (average 4.4 g); in contrast, the protein content was similar (28%) in both surveys, but, once again *verdina* accessions showed the highest protein content (> 30%). The mineral fraction of seeds (ashes content) was superior in Spanish *microsperma* type than in *macrosperma* lentils, with an overall average of 4.0 g/100 g, while the reported FAO`s values ranged 2-3.3%. In accordance with Regulation (EC) No 1924/2006 [21], the Spanish lentils of this study may be claimed as low-fat, low-sugar, very low-salt, high-protein foods and, source of minerals. The seed concentrations on macro and micronutrients are summarized in Table 7. Nitrogen content ranged from 323 to 515 mg/100 g, in accordance with the protein content. The P and K levels were quite uniform across all accessions, and in accordance with those reported by [5]. Ca, Mg, and Na were superior in *microsperma* type accessions (from Fuerteventura, León, and Granada provinces). In relation with micronutrients, the Fe content (>1g/100 g) of two accessions (from Fuerteventura Island and Granada province) excelled the reported values of 7-10 mg/100g in other surveys [3–5,23]. Mn content averaged 2.93 mg/100g, 2 times higher than the reported FAO´s values and, two *microsperma* accessions (from Fuerteventura Island and Granada) almost duplicate the average concentration. Cu content was uniform across the studied accessions, and it is in accordance with other surveys [4,5]. Zn content of *macrosperma* seeds (from Guadalajara, Ciudad Real and Lanzarote) almost duplicate the concentration on *microsperma* seeds. Most probably, these differences may be due to genetic variation among accessions rather than to soil chemical characteristics of the soil, as lentils were cultivated in the same area. The EU Regulation No 1169/2011 [17] established that when claiming a *significant amount* of a listed nutrients, the food should meet a 15 % of the nutrient reference values (NRV) supplied by 100 g. In our study, all lentils accessions can be claimed as containing significant amounts of all analyzed minerals (Table 7).

3.4. Phenolic compound in lentil accessions

Legumes have gaining additional interest because they are excellent sources of bioactive compounds and can be important sources of ingredients for uses in functional foods and other applications. A target analysis including more than 90 polyphenol compounds have been conducted in the methanolic extracts of six lentil accessions. The total number of phenolics compouns varied among accessions (Table 8). Thus, *macrosperma* type seeds with non-coloured testa had 7-9 compounds, while in *microsperma* types 10-15 compounds have been detected, as in accessions from

Fuerteventura Island, with reddish-yellow color and, accessions from León and, Granada with dark-green testa. These three accessions exclusively contain protocatechuic acid, kaempferol-3-O-glucoside, luteolin-4`-O-glucoside and, quercitin-3-O-rhamnoside, flavonoid and flavone derivatives compunds,with recognized antioxidant and anti-inflammatory activity. All accessions share 4-hydroxybenzoic acid, catechin, flavanomarein, p-cumaric acid and, salycilic acid. Gallocatechin was common in these lentils seeds but was not detected in accession from Fuerteventura Island. Both *verdina* type accessions have the highest content of phenolic compounds and, exclusively share phloretic acid, luteolin-7-O-glucoside, quercetin-4′-O-glucoside. Aromadendrene (di-hydrokaempferol) was exclusively detected in Ciudad Real lentils, it is a flavonol compound; and vanillic acid was only detected in Fuerteventura seeds. The high bioactive compounds content in coloured lentil seeds, seems a general rule in other legumes as *Phaseolus vulgaris*, *Vigna subterránea,* black soybeans and azuki beans [18,19,24] which reinforce the worldwide accepted importance of grain legumes consumption as source of bioactive compound with high antioxidative ability, in addition to their nutritional and mineral provisions.

References

1. sgapc@mapa.es Subdirección General de Análisis, Coordinación y Estadística (Accessed on 8 january 2024)
2. Poole, P.; Ramachandran, V.; Terpolilli, J. Rhizobia: From saprophytes to endosymbionts. Nat. Rev. Microbiol. **2018**, 16, 291–303. doi: 10.1038/nrmicro.2017.171
3. Langyan, S.; Yadava, P.; Khan, F.N.; Bhardwaj, R.; Tripathi, K.; Bhardwaj, V.; Bhardwaj, R.; Gautam, R.K.; Kumar, A. Nutritional and food composition survey of major pulses toward healthy, sustainable, and biofortified diets. Front. Sustain. Food Syst. **2022**, 6:878269. doi: 10.3389/fsufs.2022.878269
4. FAO/INFOODS Global food composition data base for pulses. User guide.https://www.fao.org/infoods/infoods/tables-and-databases/faoinfoods-databases. **2017** (Accessed on 15 november 2023)
5. Vandemark, G.J.; Grusak, M.A.; McGee, R.J. Mineral concentrations of chickpea and lentil cultivars and breeding lines grown in the U.S. Pacific Northwest. Crop J. **2018**, 6(3), 253-262.
6. Lázaro, A.; Ruiz, M.; de la Rosa, L.; Martín, I. Relationships between agro/morphological characters and climatic parameters in Spanish landraces of lentil (*Lens culinaris* Medik.) Genet. Resour. Crop Evol. **2001**, 48, 239–249.
7. Erskine, W. Lessons for breeders from landraces of lentil. Euphytica **1997,** 93, 107–112.
8. Bejiga G.; Tsegaye S.; Tullu A.; Erskine W. Quantitative evaluation of Ethiopian landraces of lentil (*Lens culinaris*). Genet. Resour. Crop Evol. **1996**, 43, 293–301.
9. Piergiovanni, A.R.; Laghetti, G.; Olita, G.; Monti, M.; Preiti G.; Di Prima, G. Screening for agronomic and biochemical traits in a lentil germplasm collection. In: *3rd European Conference on grain legumes proceedings*, Valladolid, Spain. November 14–19, AEP (Ed.) **1998**, pp. 206.
10. Bellucci, E.; Aguilar, O.M.; Alseekh, S.; Bett, K..; Brezeanu, C.; Cook, D.; De la Rosa, L.; Delledonne, M.; Dostatny, D. F.; Ferreira, J. J.; et al. The INCREASE project: *Intelligent collections of food-legume genetic resources for European agrofood systems*. Plant Journal. **2021**, 108(3), 646–660. https://doi.org/10.1111/tpj.15472
11. Vincent, J.M. *A Manual for the Practical Study of Root Nodule Bacteria*. Blackwell Scientific Publications, Oxford, England **1970.**
12. Beringer, J.E. R factor transfer in *Rhizobium leguminosarum* and *Rhizobium phaseoli*. J. Gen. Microbiol. **1974**, 84, 188-198.
13. Rigaud, J.; Puppo, A. Indole-3-acetic acid catabolism by soybean bacteroids. J. Gen. Microbiol. **1975**, 88, 223-228.
14. Somasegaran, P.; Hoben, H. J. *Methods in legume-Rhizobium technology*. Paia, Maui: University of Hawaii NifTAL Project and MIRCEN, Department of Agronomy and Soil Science, Hawaii Institute of Tropical Agriculture and Human Resources. USA.**1985**.
15. Albareda, M.; Rodríguez-Navarro, D.N.; Camacho, M.; Temprano, F.J. (2008) Alternatives to peat as a carrier for rhizobia inoculants: Solid and liquid formulations. Soil Biol. Biochem. **2008**, 40, 2771-2779. doi: 10.1016/j.soilbio.2008.07.021
16. Ruiz-Díez, B.; Fajardo, S.; Fernández-Pascual, M. Selection of Rhizobia from Agronomic Legumes Grown in Semiarid Soils to be Employed as Bioinoculants. Agron. J. **2012**, 104, 550-559. doi:10.2134/agronj2011.0299

 doi:10.20944/preprints202401.1074.v1

17. REGULATION (EU) No 1169/2011 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 25 October 2011 *on the provision of food information to consumers.* Oficial Journal of the European Union. **2011**.
18. Hungría, M. A.; Phillips, D.A. Effects of a seed color mutation on Rhizobial nod-gene inducing flavonoids and nodulation in common bean. M.P.M.I. **1993**, 6(4), 418-422.
19. Puozza, D.K.; Jaiswal, S.K.; Dakora F.D. Black seedcoat pigmentation is a marker for enhanced nodulation and N2 fixation in Bambara groundnut (Vigna subterranea L. Verdc.) landraces. Frontiers in Agronomy, **2021**, 3, doi.org/10.3389/fagro.2021.692238
20. Thies, J.E.; Singleton, P.W.; Bohlool, B.B. Influence of the size of indigenous Rhizobial populations on establishment and symbiotic performance of introduced rhizobia on field-grown legumes. Appl. Environ. Microbiol. **1991**, 57, 19-28.
21. REGULATION (EC) No 1924/2006 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 20 December 2006 on nutrition and health claims made on foods. Oficial Journal of the European Union. **2006**.
22. Vandemark, G.J.; Grusak, M.A.; McGee, R.J. Mineral concentrations of chickpea and lentil cultivars and breeding lines grown in the U.S. Pacific Northwest. Crop J. **2018**. https://doi:10.1016/j.cj.2017.12.003
23. Moreiras O.; Carbajal, A.; Cabrera, L.; Cuadrado, C. Tablas de composición de alimentos. Guía de Prácticas. **2013**. Piramide (Ed), Madrid, Spain.
24. Lin, P-Y; Lai, H-M. Bioactive compounds in legumes and their germinated products. J Agric. Food Chem. **2006**, 54, 3807−3814
25. van Berkum, P.; Beyene, D.; Temprano Vera, F.; Keyser, H.H. Variability among Rhizobium Strains Originating from Nodules of Vicia faba. Appl. Environ. Microbiol. **1995**, 61, 2649–2653.