



Research Article (DOI: <http://dx.doi.org/10.15580/GJAS.2014.2.021014101>)

Determination of *Phaseolin* Types in Common Bean (*Phaseolus vulgaris*) Varieties from Turkey

Seher Yıldız Madakbaş^{1*}, Halima Awale² and James Kelly²

¹Ahi Evran University, Agricultural Faculty, Department of Horticulture, Kırşehir-Turkey.

²Michigan State University, Department of Crop, Soil and Microbial Sciences, Michigan- USA.

ARTICLE INFO	ABSTRACT
Article No.: 021014101 DOI: 10.15580/GJAS.2014.2.021014101 Submitted: 10/02/2014 Accepted: 05/03/2014 Published: 13/03/2014 *Corresponding Author Abrougui K. E-mail: seheryldzmadakbas@gmail.com	The purpose of our study was to determine the phaseolin types of common bean varieties widely consumed in Turkey through protein extraction. Twenty- four bean genotypes, consisting of 22 varieties from Kırşehir province of Turkey and two control varieties (Andean and Mesoamerican) were assessed. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed with protein samples extracted from bean genotypes. Gels were stained with Coomassie blue dye R-250 and kept in fixing solution. Our analyses revealed that out of 22 bean samples, 16 contained Andean phaseolin type and 6 contained Mesoamerican phaseolin type. T-test statistical analysis was performed to determine the relationship between seed phaseolin types and seed volumes (length, height and width). The results showed that there was a significant difference between phaseolin and seed volume.

Keywords:

Phaseolus vulgaris, phaseolin, SDS-PAGE, genotype, Andean, Mesoamerican, seed volume

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is one of the most widely consumed seed based food sources around the world. It is an important legume in Turkey with its pods and seeds being consumed, although the pods are relatively more popular. Fresh common bean production in Turkey is 615,000 tons and dry bean production is 200,000 tons annually (FAO, 2011; FAO, 2012).

Aside from being a perfect nutritional source in terms of some vitamins and minerals, common bean is also rich in proteins, unsaturated fatty acids (linoleic acid) and soluble fiber (Rodiño et al., 2009). Nutritional contents in bean have therapeutic properties in cardiac and kidney diseases, several cancer types and obesity (De la Fuente et al., 2012).

Common bean contains higher levels of protein (>20%) compared to cereals such as rice and wheat (<15%) (Kaplan, 1965; Brown et al., 1981a; Chandrakanth and Hall, 2008). Phaseolin, a protein mainly stored in seeds, makes up the majority of total seed protein. It has been shown that globulin, which makes up phaseolin, consists of two major parts: vicilin and legumin. Globulin has been utilized in protein studies since total seed protein consists of 35-50% globulins (Ma and Bliss, 1978; Lioi, 1989; Rodiño et al., 2001a). However, the exact ratio varies between different bean genotypes (McLeester et al., 1973).

Phaseolin is coded by a single complex loci located in Pv07 linkage group consisting of 6-10 closely tied members of a small gene family (Brown et al., 1981b; Gepts et al., 1986; Koenig et al., 1990; Kami et al., 1995). It has been proven that phaseolin is an important and informative marker in studies on genetic diversity and evolution for both wild and cultivated lines due to its functional and structural properties (de la Fuente et al., 2012). Moreover, these characteristics provide researchers with important opportunities in determining protein compositions of bean seeds qualitatively and quantitatively towards selection for plant breeding (Gepts and Bliss, 1988; Gepts, 1993; Toro et al., 2007; Chandrakanth and Hall, 2008).

It has been shown that phaseolin types vary according to their eco-geographical origin. Two major gene pools were identified. These are Mesoamerican gene pool; from Mexico to Colombia, and Andean gene pool; from south Peru to Northwestern Argentina. (De la Fuente et al., 2012). Mesoamerican varieties have "S" (Sanilac), "M" (Middle America) or "B" (Boyacá) phaseolin types; Andean varieties have "T" (Tendergreen), "C" (Contender), "H" (Huevo de Huanchaco), "A" (Ayacudo), "J" (Jujuy) and "I" (Inca) phaseolin types. Phaseolin type "C" was reported to be a combination between types "T" and "S" of Mesoamerican and Andean origin respectively. Among cultivated varieties the most popular types are "S" and "T". Type "B" phaseolin is present only in wild and cultivated varieties of Colombia. Such variety in phaseolin types is thought to have resulted from random outcrosses in nature, contaminations from cultivation areas and multiple cultivation efforts (Brown

et al., 1981a; Gepts et al., 1986; Koenig et al., 1990; Kami et al., 1995).

Since the discovery of the Americas, common bean has been traded extensively between the old world and the new world. Common bean came to Europe in 1540s, allowing bean genotypes of Andean and Mesoamerican origin to spread around different regions of the world. It was determined that bean genotypes in Western Europe, the Iberian Peninsula and Africa are mostly of Andean origin since bean varieties in the Iberian Peninsula commonly contains type "C" phaseolin. Bean varieties of Andean origin possessed an advantage over varieties of other origins due to better photoperiodic adaptation. Among varieties of Western Europe, type "T" phaseolin is the most dominant because of favored cultivation due to their green pods (Rodiño et al., 2001a). Genotypes originating from Andean mountains have adapted to cold European summers better than Mesoamerican genotypes. Thousands of local genotypes of Andean and Mesoamerican gene pools have been cultivated in Europe and preserved in seed banks.

Researchers have utilized molecular markers to determine genetic structures and origins of bean varieties in addition to the seed storage protein "phaseolin" (Piergiovanni et al., 2000; Rodiño et al., 2003; Logozzo et al., 2007; Limongelli et al., 1996; Perez-Vega et al., 2009; Maras and Sustar-Vozlic, 2013). Polyacrylamide gels have usually yielded the best results in determining phaseolin types. Analysis with sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) yields highly stable bands. Thus, types of phaseolin have been identified more easily according to their molecular weight (53kD – 20kD) (Derbyshire et al., 1976; Lioi, 1989).

In this study, the objectives were: 1) pave the way for future studies aiming to determine the structure of phaseolin seed storage protein by determining variability of phaseolin types among common bean genotypes in Turkey, 2) create a preliminary model for phylogenetic studies and 3) assist breeding programs by determining genetic relationships between common bean genotypes.

MATERIALS AND METHODS

Seeds of bean samples

Twenty-two common bean genotypes were provided by the project called: "Characterization of Local Bean Populations in Kırşehir Province" conducted in Kırşehir Ahi Evran University, Faculty of Agriculture, Department of Horticulture in year 2012. Two control genotypes (G 19833-Andean and Dor 364 Mesoamerican) were provided by Michigan State University, Plant Soil and Microbial Sciences Department. Turkish genotypes were G 88-10, G 62, G 68, G 111, G 11, G 28, G 106, G 19, G 70, G 108, G 77, G 105, G 104, G 110, G 16, G 17, G 112, G 64, G 32, G 69, G 35 and G 107.

Protein extraction from seeds

The seed coats from three seeds of each genotype were peeled and the cotyledons were homogenized in 2000 Geno/Grinder (Spex/Ception) for five minutes at 800 rpm. Fifty milligrams of bean flour was measured and placed into 1.5mL Eppendorf tube. 500 μ L of Protein Extraction Buffer (0.036 M Tris-HCl pH 8.5) was added and the tubes were gently mixed. Samples were held inside the sonication chamber containing ice and water for one hour and centrifuged (Microcentrifuges Eppendorf Refrigerated 5418R) at 14,000 rpm for 20 minutes. 400 μ L of supernatant was transferred into fresh tubes. Cracking buffer with SDS (Tris-HCl 0.625M pH 6.8) was added to the supernatant at 1:1 v/v ratio. Samples were vortexed for two minutes, incubated in 100°C water bath for five minutes and stored at -20°C after being cooled to room temperature for long-term storage (Toro et al., 2007; Ligarreto and Ocampo, 2012).

Electrophoresis

Electrophoresis was performed after loading samples into NuPAGE 10% Bis-Tris mini gel – NOVEX (provided by Life technologies). Miniprote in Xcell Unit and BIO-RAD SDS-PAGE markers (20kD-103kD) were used. 1 μ L from samples and 5 μ L from markers were loaded. Electrophoresis was performed at 110V and 80mA for 80 minutes. Following electrophoresis gels were incubated in Coomassie blue R-250 Buffer at 100 rpm for 1 hour in an orbital shaker. Fixing was performed by incubating the gels in 50ml of fixing solution. The solution was replaced with fresh fixing solution every hour for 3-4 hours (Laemmli, 1970; Bradford, 1976; Ma and Bliss, 1978; Lareo et al., 1993; Rodino et al., 2001b).

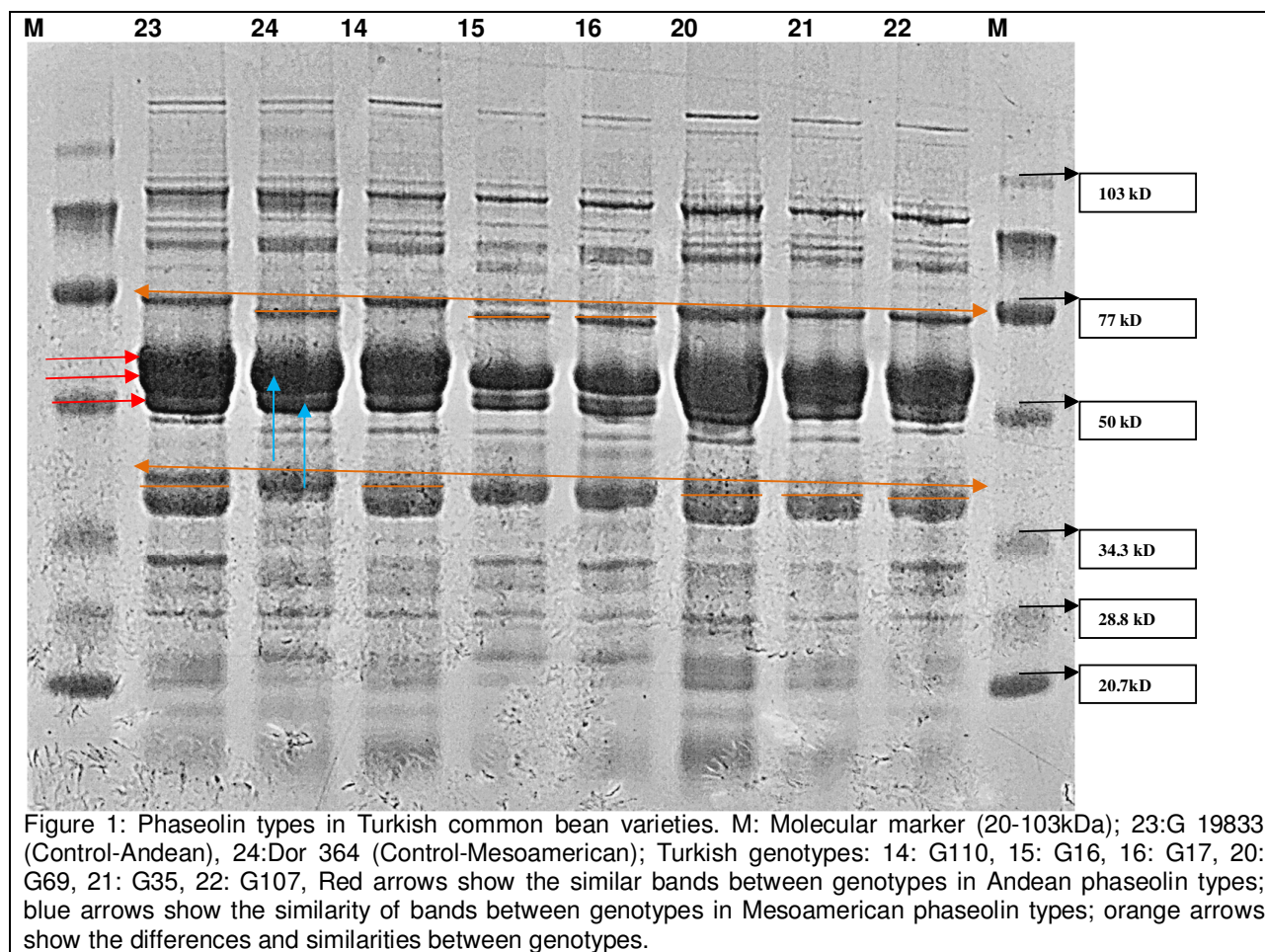
Seed volume

Phaseolin types and seed volumes (length, height and width) were determined as performed by Gepts and Bliss (1986). Correlations between phaseolin type and seed volume were determined by Mann-Whitney U Test, the non-parametric equivalent of T-test statistical analysis (Stonehouse and Forrester, 1998).

RESULTS AND DISCUSSION

Twenty-two common bean genotypes and two control varieties were analyzed on SDS-PAGE gels along with

a molecular marker (20kD-103kD). Differences in phaseolin types between samples and control varieties are shown in Figure 1 in detail. Band sizes and the number of bands were different between control and sample varieties. Three bands between 49kD and 55kD were observed for Andean genotypes, while for Mesoamerican genotypes two bands between 50kD and 53kD was observed. The number of the bands observed in a specific region of the gel may vary according to the relative weights of the proteins the sample contains. After these bands were highlighted by Coomassie Blue, sharp and proper bands were also obtained along with large and wide bands. This has been reported as a result of different sized seed samples being used in protein extractions (McLeester et al., 1973), which was confirmed in the gel images we obtained. As can be seen in Figure 1, bean genotypes 14 (G110), 20 (G69), 21 (G35) and 22 (G107) have produced thick bands whereas genotypes 15 (G16) and 16 (G17) have produced proper bands. Such bands were also obtained in other gel images not provided here. Although Phaseolin protein has a narrow range of molecular weight (45-52 kD), phaseolin has been reported to have various molecular weights by different researchers. Mcleester *et al.* (1973) reported phaseolin having a molecular weight of 53, 47 and 43 kD; Derbyshire et al. (1976) reported 49, 45 and 42 kD; Bollini and Chrispeels (1978) reported 52 and 49 kD (Ahn et al., 1991). The molecular weights of the bands obtained in this study correlates with those reported by other researchers. However the method of extraction and characterization of phaseolin can lead to different results obtained by different laboratories (Derbyshire et al., 1976). SDS-PAGE analysis of phaseolin has been utilized worldwide as an effective tool in analyzing genetic variability, evolutionary relationships and correlation between molecular weight of the band and genotype among bean populations (Brown et al., 1981b; Mcleester et al., 1973; Romero et al., 1975; Gepts, 1998; Singh, 2001; Rodiño et al., 2001b). We have also observed that SDS-PAGE analysis was useful in tracking the origins of bean genotypes that have been dispersed into various regions because PAGE analyses revealed any changes in genetic variability during the cultivation process. These changes in the Phaseolin locus (Phs) have been shown in Mesoamerican genotypes rather than Andean genotypes (Gepts, 1993; Kami et al., 1995; Chacon et al., 2005).



Phaseolin type, seed volume and color are shown in Table 1. It was determined that out of 22 Turkish bean genotypes, 16 had Andean type phaseolin and 6 had Mesoamerican type phaseolin. Studies on phaseolin and its allozymes have shown that the Andean type phaseolin is the predominant type in worldwide (Singh et al., 1991; Rodiño et al., 2001; Santalla et al., 2002; Rodiño et al., 2006). Sustar-Vozlic et al. (2006) have identified Europe as the second center of domestication of the Andean gene pool since the European bean germplasm dates back to 200 years ago. They reported that European accessions have great morphological similarities with the Andean bean genotypes obtained from Chile. Angioi et al. (2010) indicated that Andean genotypes have a greater effect on European accessions compared to Mesoamerican genotypes. European accessions were determined to contain 72% "T", 21% "S" and 7% "C" type phaseolin and accessions of Iberian Peninsula to contain 43% "C", 29% "S" and 1% "H" phaseolin. The reason for this is that genotypes of Andean origin are more adapted to ecological conditions compared to Mesoamerican genotypes. Moreover, Andean genotypes containing "T" type phaseolin are preferred in Europe and Turkey because of their green pods which are consumed as fresh bean. It has been reported that among the genotypes consumed as fresh beans, 66% had "T",

25% had "S" and 9% had "C" type phaseolin (Maras and Sustar-Vozlic, 2013).

In our study we have used T-test statistical analysis to determine the correlation between phaseolin types and seed volumes. Analyses have shown that there is significant difference between phaseolin types in terms of seed volumes (Table 2). Seeds containing Andean type phaseolin were determined to be larger compared to those containing Mesoamerican type phaseolin. Phaseolin alleles were determined to be correlated with seed size and the seed sizes were under control of a quantitative loci (Perez-Vega et al., 2009). Other than phaseolin types, the seed volumes varied among genotypes. Previous studies have shown that "T", "C", "H" and "A" (Andean) phaseolin types were associated with larger and wider seeds compared to "S" and "B" (Mesoamerican) genotypes (Gepts et al., 1986; Gepts and Bliss, 1986; Rodiño et al., 2009).

As shown in Table 1, seeds from Mesoamerican genotypes had dark maroon, dark purple or white color whereas Andean genotypes showed great variability. Morphological analysis of the seeds has shown that small white and red seeds had type "S" phaseolin of Mesoamerican origin, seeds with dark spots on cream background had type "T" phaseolin of Andean origin, seeds with zebra stripes pattern on cream background had type "C" phaseolin

and wide kidney shaped seeds with dark maroon-purple and cream seeds containing red veins had both type "T" and "C" phaseolin. White seeds were observed in both Mesoamerican and Andean genotypes, but seeds from the Mesoamerican gene pool showed to be smaller than seeds from the Andean gene pool (Gepts and Bliss, 1988). The analyses we have performed have shown that white seeds of both Andean and Mesoamerican origin had variability in terms of size. The data on seed color with Mesoamerican and Andean origin matches with the provided in the literature. Among these genotypes G 106, G 110 (Andean) and G 11 (Mesoamerican) had an almost black dark purple color. Seeds of control

variety DOR-364 (Mesoamerican) were also of the same color. The shared seed color was reported to be resulting from recombination between Andean and Mesoamerican genotypes (Singh et al., 1991; Acquaah and Isleib, 1994; Santalla et al., 2002; Rodino et al., 2006).

Studies on phaseolin type determination aid researchers in understanding wide dispersal of bean genotypes from their original location to their secondary location (De la Fuente et al., 2012). Bean cultivation has been widespread through the Mediterranean region and great variation has occurred. This occurred as a result of different preferences of farmers in various locations.

Table1: Phaseolin types, seed volumes and seed colors of Turkish common bean genotypes and control varieties

Genotype No	Genotype	Phaseolin type	Seed volume (mm)			Seed color
			Length	Width	Height	
1	G88-10	A	13.70	8.42	6.82	Maroon stripes on dark cream background
2	G62	A	13.91	8.03	6.32	Red stripes on light cream background
3	G68	MA	9.15	5.63	4.93	Dark maroon
4	G111	MA	9.29	6.52	4.97	Dark maroon
5	G11	MA	10.32	8.07	5.47	Deep purple
6	G28	A	13.21	6.75	5.72	Light brown
7	G106	A	11.90	7.60	6.36	Deep purple
8	G19	A	13.19	8.42	7.55	Brown stripes on brown background
9	G70	A	13.73	7.36	5.15	Light maroon
10	G108	A	12.12	7.69	5.17	White
11	G77	A	15.74	10.13	8.00	Dark brown strips on brown background
12	G105	A	15.82	7.46	5.73	Red veins on light brown background
13	G104	A	14.93	8.79	6.60	Dark maroon
14	G110	A	14.40	8.92	6.44	Deep purple
15	G16	MA	10.55	7.05	5.45	White
16	G17	MA	9.19	5.72	4.75	White
17	G112	A	13.20	10.85	6.93	Maroon
18	G64	A	13.81	7.71	6.92	Occasional veins on light reddish brown background
19	G32	MA	10.71	6.67	3.64	White
20	G69	A	14.60	6.31	4.66	Deep maroon stripes on cream background
21	G35	A	13.82	7.47	6.35	Dark maroon
22	G107	A	16.09	9.42	7.74	Maroon stripes on cream background
23	G19833 (C) ^a	A	13.90	7.20	5.33	Dark maroon strips on dark reddish brown background
24	Dor364 (C) ^a	MA	9.18	6.26	4.78	Very deep purple

^aC: Control

Table 2: Phaseolin types and seed volumes

Types of phaseolin	Number genotype according to Phaseolin types	Seed size (mm)		
		length	width	height
A	17	16.00	8.14	6.34
MA	7	4.00	6.56	4.85
T-test	24	3.779**	3.212**	3.784**

** significant at the 0.01 level; A:Andean; MA: Mesoamerican

Phaseolin variability in countries around the Mediterranean region, including Turkey, can be compared to Central and South American countries (Gepts et al., 1986; Lioi, 1989; Chacon et al., 2005). It was reported that 44% of European bean germplasm consists of hybridizations between Andean and Mesoamerican gene pools (Angioi et al., 2010; Santalla et al., 2002). Rodino et al. (2006) have stated that hybrid genotypes have formed a secondary center for genetic variability in bean because wild ancestral genotypes have formed the genetic basis for today's local and cultivated varieties.

CONCLUSIONS

In this study, we have identified phaseolin types in common bean genotypes by protein extraction and SDS-PAGE analysis. However this work helped in the identification of phaseolin types in Turkey in order to reveal the genetic variability in the germplasm since data on current bean collections is limited. In future studies on phaseolin types, the number of seeds has to be high enough to represent the present variability. This study will be a preliminary model for phaseolin studies in Turkey.

ACKNOWLEDGEMENTS

This study was conducted in Michigan State University, Plant Soil and Microbial Sciences Department, Bean Genetics Lab in year, 2013. We would like to thank Cesar Umberto Ocampo, Caroline Astudillo-Reyes, Hussein Alameldin, Mete Gurhan Dasci and Asli Akilli for their assistance in this study.

LITERATURE CITED

- Acquaah G, Isleib TG (1994). Gene pool specificity, paucity of enzyme variation and phaseolin polymorphism in the common bean. *HortScience*. 29:1337–1339.
- Ahn J, Sen CL, Whitaker JR (1991). Stability of tertiary structure of Phaseolin of Red Kidney Bean (*Phaseolus vulgaris*) as limiting factor in proteolysis. *Journal of Food Biochemistry*. 15: 263–278.
- Angioi SA, Rau D, Attene , Nanni L, Bellucci E, Logozzo G, Negri V, Spagnoletti Zeuli PL, Papa R, (2010). Beans in Europe: origin and structure of the European landraces of *Phaseolus vulgaris* L. *Theor Appl Genet*. 121:829–843.
- Bollini R, Chrispeels MJ (1978). Characterization and subcellular localization of vicilin and phytohemagglutinin, the two major reserve proteins of *Phaseolus vulgaris* L. *Planta*. 142: 291–298.
- Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*. 72: 248–254.
- Brown JWS, Bliss FA Hall TC (1981a). Linkage relationships between genes controlling seed proteins in French bean. *Theor Appl Genet* 60: 251–259.
- Brown JWS, Ma Y, Bliss FA Hall TC (1981b). Genetic variation in the subunits of globulin-1 storage protein of French bean. *Theor Appl Genet* 59: 83–88.
- Chacon MI, Pickersgill SB, Debouck DG (2005). Domestication patterns in common bean (*Phaseolus vulgaris* L.) and the origin of the Mesoamerican and Andean cultivated races. *Theor Appl Genet*. 110: 432–444.
- Chandrakanth E, Hall TC (2008). Phaseolin: structure and evolution. *The Open Evolution Journal*. 2: 66–74.
- De la Fuente M, María López-Pedrous M, Alonso J, Santalla M, de Ron A., Álvarez G, Zapata C (2012). In-depth characterization of the Phaseolin protein diversity of Common Bean (*Phaseolus vulgaris* L.) based on two-dimensional electrophoresis and mass spectrometry. *Food Technol Biotechnol*. 50 (3): 315–325.
- Derbyshire E, Wright D J, Boulter D (1976). Legumin and Vicilin, storage proteins of legume seeds. *Phytochemistry*. 15: 3–24.
- FAO (2011). www.fao.stat.fao.org
- FAO (2012). www.fao.stat.fao.org
- Gepts P, Bliss F A (1986). Phaseolin variability among wild and cultivated Common Beans (*Phaseolus vulgaris*) from Colombia. *Economic Botany*. 40(4): 469–478.
- Gepts P, Osborn TC, Rashka K, Bliss FA (1986). Phaseolin-protein variability in wild forms and landraces of the common bean (*Phaseolus vulgaris*): evidence for multiple centers of domestication. *Econ Bot*. 40: 451–468.
- Gepts P, Bliss FA, (1988). Dissemination pathways of Common Bean (*Phaseolus vulgaris*, Fabaceae) deduced from Phaseolin electrophoretic variability. II. Europe and Africa. *Economic Botany*. 42(1): 86–104.
- Gepts P (1993). The use of molecular and biochemical markers in crop evolution studies. *Evol Biol*. 27: 51–94.
- Gepts P (1998). Phaseolin as an evolutionary marker. In: genetic resources of *Phaseolus* beans, P. Gepts (Ed.), Kluwer Academic Publishers, Dordrecht, The Netherlands, 215–241.
- Kaplan L (1965). Archeology and domestication in American *Phaseolus* (beans). *Econ Bot*. 19: 358–368.
- Kawi J, Velasquez VB, Debouck GD, Gepts P (1995). Identification of presumed ancestral DNA sequences of phaseolin in *Phaseolus vulgaris*. *Proc Natl Acad Sci*. 92, 1101–1104.
- Koenig RL, Singh SP Gepts P (1990). Novel phaseolin types in wild and cultivated common bean (*Phaseolus vulgaris*, Fabaceae). *Econ Bot*. 44: 50–60.
- Laemmli VK (1970). Cleavage of structure proteins during assembly of the head of bacteriophage T4. *Nature*. 22: 680–685.

- Lareo LR, Gonzalez AV, Barona E, Beebe S (1993). Fast and reliable one and two-dimensional electrophoretic parameters for phaseolin type identification. *Annu Rept Bean Improvement Coop (USA)*. 36: 8-9.
- Ligarreto G, Ocampo C (2012). Genetic diversity in a Colombian bean (*Phaseolus vulgaris* L.) collection as assessed by phaseolin patterns and isoenzymatic markers. *Agronomía Colombiana*. 30(2): 179-187.
- Limongelli G, Laghetti G, Perrino P, Piergiovanni AR (1996). Variation of seed storage proteins in landraces of common bean (*Phaseolus vulgaris* L.) from Basilicata, Southern Italy. *Euphytica*. 92: 393-399.
- Lioi L (1989). Variation of the storage protein phaseolin in common bean (*Phaseolus vulgaris* L.) from the Mediterranean area. *Euphytica*. 44 : 151 -155.
- Logozzo G, Donnoli R, Macaluso L, Papa R, Knupffer H, Spagnoletti Zeuli PL (2007). Analysis of the contribution of Mesoamerican and Andean gene pools to European common bean (*Phaseolus vulgaris* L.) germplasm and strategies to establish a core collection. *Genet Resources Crop Evol*. 54:1763-1779.
- Ma Y, Bliss F (1978). Seed proteins of common bean. *Crop Sci*. 18: 431-437.
- Maras M, Sustar-Vozlic J (2013). Genetic diversity and dissemination pathways of Common Bean in Central Europe. *J Amer Soc Hort Sci*. 138(4):297-305.
- Mcleester RC, Hall T C, Sun SM, Bliss F- A (1973). Comparison of globulin proteins from *Phaseolus vulgaris* with those from *Vicia faba*. *Phytochemistry*. 2:85- 93.
- Perez-Vega E, Campa A, De la Rosa L, Giraldez R, Ferreira JJ (2009). Genetic diversity in a core collection established from the main bean genebank in Spain. *Crop Sci*. 49:1377-1386.
- Piergiovanni AR, Taranto G. and Pignone D. (2000). Diversity among common bean populations from the Abruzzo region (Central Italy): a preliminary inquiry. *Genet Resources Crop Evol* 47:467-470.
- Rodiño P, Monteagudo AB, Santalla M (2001). Methodology for electrophoretic analysis of Phaseolin. Ed: Jacobsen HJ, Muzquiz M, Hassa A, Handbook on Common Bean Related Laboratory Methods. PHASELIEU - FAIR - PL97-3463, Misión Biológica de Galicia (CSIC).
- Rodiño AP, Santalla M, Montero I, Casquero PA de Ron AM (2001). Diversity in common bean (*Phaseolus vulgaris* L.) germplasm from Portugal. *Genet Resour Crop Evol*. 48: 409-417.
- Rodiño P, Santalla M, de Ron AM, Singh SP (2003). A core collection of common bean from the Iberian Peninsula. *Euphytica*. 131:165-175.
- Rodiño AP, Gonzalez AM, Santalla M, De Ron AM Singh SP (2006). Novel genetic variation of the common bean in Europe. *Crop Sci*. 46:2540-2546.
- Rodiño A P, Monteagudo AB, de Ron A M Santalla M (2009). Ancestral landraces of common bean from the South of Europe and their agronomical value for breeding programs. *Crop Science*. 49:2087-2099.
- Romero J, Sun SM, McLeester RC, Bliss FA, Hall TC (1975). Heritable variation in a polypeptide subunit of the major storage protein of the bean (*Phaseolus vulgaris* L.). *Plant Physiol*. 56:776-779.
- Santalla M, Rodino AP, de Ron AM (2002). Allozyme evidence supporting southwestern Europe as a secondary center of genetic diversity for the common bean. *Theor Appl Genet*. 104: 934-944.
- Singh SP, Gutierrez JA, Molina A, Urrea C, Gepts P (1991). Genetic diversity in cultivated common bean. II. Marker-based analysis of morphological and agronomic traits. *Crop Sci*. 31:23-29.
- Singh SP (2001). Broadening the genetic base of common bean cultivars: A review. *Crop Sci*. 41:1659-1675.
- Stonehouse J M, Forrester GJ (1998). Robustness of the t and U tests under combined assumption violations. *Journal of Applied Statistics*. 25: 63-74.
- Sustar-Vozlic J, Maras M., Javornik B, Meglic V (2006). Genetic diversity and origin of Slovene common bean (*Phaseolus vulgaris* L.) germplasm as revealed by AFLP markers and phaseolin analysis. *J Amer Soc Hort Sci*. 131:242-249.
- Toro O, Ocampo CH., Debouck DG (2007). Phaseolin: variability and reference materials in wild and cultivated common bean. *Annu Rept Bean Improvement Coop (USA)*. 50: 69-70.