Acta agriculturae Slovenica, 90(november 2007)1, 33–43.

http://aas.bf.uni-lj.si

Agris category codes: F30 COBISS Code 1.01

GENETIC VARIABILITY OF SOME QUALITY TRAITS IN *Lathyrus* spp. GERMPLASM

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Received September 27, 2007, accepted October 15, 2007.
Delo je prispelo 27. septembra 2007, sprejeto 15. oktobra 2007.

ABSTRACT

Sixty-six accessions representing eighteen species of the genus *Lathyrus* collected from different geographic regions were evaluated for variations of quality traits (100 seeds weight, ash, total seed proteins and 3-(-N-oxayl)-L-2,3 diaminopropoinc acid – ODAP contents). High variability of ODAP levels was exhibited at both inter-specific and intra-specific levels. This variability was attributed to genetic and environmental factors. No significant correlation was found between ODAP and each of total protein content, ash content and 100 seeds weight. Cluster analysis of C.V. (Coefficient of variance) values for each accession identified the sixty-six accessions into eight groups. The most promising accession for breeding programs was *L. sativus* from Tunisia. This accession has good grain quality due to relatively low ODAP level and high protein content. The variations of protein content, ash content and 100-seeds weight were also discussed.

Key words: legumes / *Lathyrus sativus* / ODAP / genetic variability / quality traits / germplasm

GENETSKA VARIABILNOST NEKATERIH PARAMETROV KAKOVOSTI SEMENA *Lathyrus* spp.

IZVLEČEK

Ocenili smo variabilnost kakovosti (masa 100 semen, pepel, skupne beljakovine in vsebnost 3-(-N-oksail)-L-2,3 diaminoproposke kisline – ODAP) 66 akcesij 18 vrst rodu *Lathyrus*, ki smo jih zbrali z različnih geografskih regij. Ugotovili smo visoko stopnjo variabilnosti vsebnosti ODAP med vrstami in znotraj njih. To variabilnost povzročajo tako genetsko kot okoljski dejavniki. Nobene značilne povezave nismo našli med vsebnostjo ODAP in vsebnostjo celokupnih beljakovin, vsebnostjo pepela in maso 100 semen. Klasterska analiza variacijskega koeficienta (C.V.) je 66 akcesij razdelila v osem skupin. Najbolj obetavna akcesija za nadaljnje žlahtnjenje je bila akcesija *L. sativus* iz Tunizije. Ta akcesija ima dobro kakovost zrna, ki je posledica nizke vsebnosti ODAP in visoke vsebnosti beljakovin. Presojali smo tudi variabilnost vsebnosti beljakovin, pepela in mase 100 semen.

Ključne besede: stročnice / *Lathyrus sativus* / ODAP / genetska variabilnost / lastnosti / kakovost semena

INTRODUCTION

Lathyrus (*Leguminosae*; *Papilionoideae*) is the largest genus in tribe *Vicieae* and has an importance as traditional foodstuffs in many cultures worldwide (Kenicer *et al.,* 2005). It is a very popular crop in many Asain and African countries where it is grown either for stockfeed or human consumption. Kislev (1989) reported that the domestication of *Lathyrus* began in the Balkan Peninsula as a consequence of the Near East agriculture expansion into the region. Now the cultivation of *Lathyrus* spread to include margainal lands in Syria, Lebanon, Egypte, Libya, Alegria, Morocco, France and Spain, the Mediterranean basin.

The most importance traits of *Lathyrus* consists of drought tolerance, resistance of stored grains to pests, adaptability to nearly all type of soils as well as to adverse climatic conditions and low input enviroment (Abdel Moneim *et al*., 1999; Hanbury *et al*., 1999; Sharma *et al*., 2000; Granati *et al*., 2003)

In spite of the importance of *Lathyrus* for human and animal, it has a limited uses due to the presence of the neurotoxic compound 3-(-N-oxayl)-L-2,3 diaminopropoinc acid – ODAP contents. The other forms of the toxic substances in *L. sativus* are BOAA (Beta-N-oxalyl amino-L- Alanine) (Smartt *et al*., 1994; Williams *et al*., 1994). The neurotoxin compound causes an irreversible paralysis of the lower limbs in human and the four limbs in animal and is known as Lathyrism (Spencer *et al*., 1986; Williams *et al*., 1994). Lathyrism has been known to occur in grasspea areas of the world for a long time. The disease was recorded first in the Narowal area of district Sialkot.

Neurotoxin concentration is lower in *L. cicera* than in *L. sativus*. In *L. cicera* it ranges from 0.04–0.76%. These values are genotype-dependent and show a little environment interaction (Hanbury *et al*., 2000). Campbell and Briggs (1987) reported that *L. sativus* Var. 8246 has low ODAP content ranging from 0.0259 to 0.0401% (w/w) of dry seed. The safe content of ODAP for human consumption is lower than 0.2% (Abdel Moneim *et al*., 1999).

Due to the neurtoxin presence, *Lathyrus* product has been banned in many countries. However, due to the importance of this crops in developing countries, these countries has established of breeding programmes mainly focused on getting a genotype with high seed yeild and low toxicity. Hanbury *et al.* (1995) and Granati *et al.* (2003) found a considerable variation in the neurotoxin content in *L. sativus* germplasm of different origin.

There are two ways to have a genotype with high seed yield and low toxicity, first is using the genetic engineering to produce transgenic plants (Hanbury *et al*., 1999; Hanbury *et al*., 2000). The second way is through eliminate the toxic substance by careful selection so far and through hybridization between low and high toxin varieties (Qayyum *et al*., 2001; Ben Brahim *et al*., 2001). To follow such way, enough information about genetic diversity and genetic resources of *Lathyrus* germplasm around the world is needed. This can be done through studying the variations in many genetically based traits among *Lathyrus* populations such as morphology, taxonomy and molecular markers.

A series of studies adovcated to ascertain the genetic variability present in *Lathyrus* germplasm of different origin (Infantino *et al*., 1994; Granati *et al*., 2000; Alba *et al*., 2001; Polignano *et al*., 2001). What has been done of the collected germplasm around the world is not a heck of a lot. This work, therefore, advocated to evaluate the genetic variability of 100 seeds weight, and total seed protein, ash and ODAP contents in 66 accessions belong to 18 species of different origins. The selection for recovering the accessions with superior quality traits introduces a valuable genetic material for local or national breeding programmes

MATERIAL AND METHODS

Material

Seeds of *Lathyrus spp* were obtained as a donation from International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria and germplasm collection of the USDA, ARS, WRPIS Washington State University, Regional Plant Introduction Station, 59 Johnson Hall, P.O. 646402 Pullman, Washington, United States, 99164-6402.

Methods

Quantitative estimation of total seed proteins

Total proteins were extracted separately from 20 mg air-dried defatted seed meals in 1000 µl extraction buffer (0.125 µ Tris borate pH 8.9, 1% SDS) for 24 hours at –4 °C. After that time, the extracts were centrifuged for 10 minutes at 1000 g. Quantitative estimation of the total seed proteins was made according to Bradford (1976). Three replicas were made for each sample.

Ash content and 100 seeds-weight

The ash content was determined by combusting seeds in silica crucibles in a muffle furnace at 550 °C for 6 h, with 50% of the ash free mass being regarded as carbon content (Allen *et al.*, 1974). In addition, for each accession 100-seeds weight (g) was recorded.

ODAP content

Neurotoxin level was analyzed following a modified Rao's procedure (Rao, 1987). The seeds were finely milled and 100 mg of the grass pea flour were extracted for 5 h with 10 ml ethanol 60% (v/v). The suspension was then centrifuged and 75 µl of the supernatant were added to 92 µl of distilled water and 0.33 ml of 3N KOH. The sample (4 replica/genotype) was kept in a boiling water bath for 30 min (alkaline hydrolysis to convert from ODAP to DAP which can be determined) and then brought to 1 ml with water.

To detect ODAP, OPT (Ortho-phathalaldehyde) reagent was used, it was composed of 100 mg of OPT, 1ml 95% ethanol, 0.2 ml of mercaptoethanol and 99 ml of potassium tetra borate buffer (0.05 M in distilled water, pH 9.9). This reagent freshly made and used for 3 days long only. OPT reagent (2 ml) was added to the sample and absorbance of resulting yellow solution was measured after 30 min using a spectrophotometer set at λ = 420 nm . The results obeyed Beer's law.

$$\mathbf{A = C\,\varepsilon\,L}$$

(A) absorbance, (C) concentration, (ε) extinction co-efficient absorbitinty is constant and (L) is the path length which always be 1 cm.

Data analysis

The genetic diversity among the populations was evaluated by the Jaccard similarity index, Regression analysis, co-efficient of variance (CV). Multivariate analysis (factor analyses and cluster analysis) were made using the software package »SYSTAT for Windows«, Version 7.0 copyright (C) 1997, SPSS INC. A dendrogram was constructed through the complete linkage-joining rule.

RESULTS

Means, standard errors, range of variation estimated for each trait in all accessions are reported in Table 1. Protein content ranged from 22.6 to 49.3% with mean value of 35.4%. Extreme ODAP levels were 0.19 and 6.2% showing mean value of 1.05%. One hundred seeds weight was more variable, ranging from 1.35 to 31.5 g. On the contrary, the ash content showed variation ranging from 1.2 to 8.6%.

Table 1. Mean values, standard error (SE), ranges (Max, Min) and, coefficients of variation (C.V.) observed in 66 accessions belonging to 18 species of *Lathyrus* spp.
Preglednica 1. Srednje vrednosti, standardna napaka (SE), interval (maksimum, minimum) in koeficient variabilnosti (C.V.) pri 66 akcesijah 18 vrst *Lathyrus* spp.

Trait	Mean ± SE	Max	Min	C.V.
Protein content, mg g^{-1} dry matter)	354.166 ± 53.091	493.680	226.440	0.150
Ash content, %	5.147 ± 8.089	8.6000	1.200	1.571
100 Seeds Weight, g	8.472 ± 6.019	31.530	1.354	0.710
ODAP content, %	1.051 ±1.036	6.230	0.190	0.985

K-means clustering separated the 66 accessions into 8 clusters (Table 2). Cluster memberships, means, minimum and maximum values for each cluster are presented in Table 2. Clusters included different proportions of accessions belong to different species and from different countries, except cluster 2, 5 and 6 were homogenous in terms of the species they belong to. In particular, cluster 1 included accessions belong to *L. aphacce, L. sphaerius, L. hirsutus, L. sylvestris, L. hierosolymitan, L. gorgoni* and *L. inconspicuos* and showed the highest mean value for the total protein content. Clusters 2 and 6, the smallest clusters contained one accession each, included accession of *L. sylvestris* originated in Germany and accession of *L. sativus* originated in Tunisia respectively. It was interesting to note that cluster 2 presented the highest mean value for ash content and ODAP, and cluster 6 the highest mean value for 100 seed weight. Cluster 7, the most interesting one, showed the lowest mean value for ODAP (0.54) and intermediate values for the other traits. The low ODAP accessions were represented by the accession of *L. inconspicuos* collected from Turkey.

Table 2. Cluster memberships, cluster mean, coefficient of variation, and minimum and maximum values
Preglednica 2. Sestava klastrov, srednje vrednosti, koeficient variabilnosti ter najmanjše in največje vrednosti

Sample	TPC, mg g^{-1}	Ash content, %	Wt 100 seed, g	ODAP, %
L. aphacce Syria	364.100	3.300	3.552	0.3
L. aphacce Iran	416.160	4.500	1.525	0.24
L. sphaerius Syria	369.240	1.200	2.242	0.32
L. sphaerius Turky	363.120	1.900	1.767	0.35
L. hirsutus Egypt	399.840	4.300	2.900	0.320
L. hirsutus France	471.240	4.800	2.151	0.685
L. hirsutus Turky	412.060	3.800	2.264	0.470
L. sylvestris USA	493.680	5.400	4.117	6.230
L. sylvestris Yugoslavia	424.320	4.800	3.350	3.580
L. sylvestris Kazakhstain	440.640	5.500	3.975	3.970
L. hierosolymitan Turky	371.280	2.400	4.014	0.259
L. gorgoni Turky	367.200	4.500	2.893	0.335
L. inconspicuos Yughoslvia	252.960	1.800	1.354	0.3
Mean ± SE	395.83 ± 60.13	3.71 ±1.45	2.78 ± 0.97	1.34 ± 1.95
Min	252.96	1.20	1.35	0.24
Max	493.68	5.50	4.12	6.23
C.V	0.152	0.390	0.348	1.455

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Sample	TPC, mg g^{-1}	Ash content, %	Wt 100 seed, g	ODAP, %
L. sylvestris Germany	391.68	7.40	3.427	2.070
Mean ± SE	391.68	7.40	3.43	2.070
Min	391.68	7.40	3.43	2.070
Max	391.68	7.40	3.43	2.070
C.V	0.00	0.00	0.00	0.00
L. sativus Ethiopia	291.720	5.800	7.720	1.400
L. sativus Bangladish	346.800	3.900	8.139	0.560
L. sativus India	365.160	3.700	7.970	1.460
L. clymenum AUS	367.200	5.270	7.666	1.31
L. clymenum Portugal	361.080	4.080	7.632	0.82
L. clymenum Turky	363.120	3.100	7.307	0.33
L. latifolius Netherland	401.880	3.600	5.845	0.3
L. tingitanus Portugal	373.320	7.010	9.443	0.28
L. annus Turky	395.800	3.060	6.479	1.64
L. sylvestris Moroco	422.280	8.600	8.452	0.820
L. hierosolymitan Plastine	344.760	2.200	7.036	0.270
L. gorgoni Jordon	373.320	4.200	6.142	0.396
L. articulatus Grc	352.920	3.900	7.308	0.830
L. articulatus Frc	354.960	1.900	7.000	0.890
L. articulatus Austalia	361.080	1.700	7.063	0.600
L. mamoratus Turky	367.200	2.900	7.300	0.612
L. ciceria Pakistan	361.080	4.900	7.541	1.790
L. ciceria Portugal	242.760	6.500	8.819	0.94
Mean ± SE	358.14 ± 39.29	4.24 ± 1.84	7.49 ± 0.89	0.85 ± 0.49
Min	242.76	1.70	5.84	0.27
Max	422.28	8.60	9.44	1.79
C.V	0.110	0.349	0.120	0.576
L. sativus Egypt	340.680	3.900	14.180	0.850
L. sativus Ussr	316.187	3.300	13.630	1.410
L. sativus Afghanstan	318.240	2.600	11.980	1.060
L. sativus Germany	330.480	3.900	16.090	0.330
L. sativus Italy	408.000	4.200	19.880	0.830
L. sativus Yughoslavia	346.800	3.400	13.390	1.380
L. sativus Canda	328.440	6.900	17.240	0.914
L. ochrus Portugal	336.600	3.600	15.426	1.400
L. pseudocicer Jordon	269.280	3.040	9.974	0.910
L. blepharicar Turky	275.400	3.300	9.872	0.304
Mean ± SE	327.01 ±38.64	3.81 ± 1.18	14.17 ± 3.15	0.94 ± 0.40
Min	269.28	2.60	9.87	0.30
Max	408.00	6.90	19.88	1.41
C.V	0.12	0.310	0.222	0.425
L. sativus Libia	373.320	2.800	23.400	1.530
L. sativus Spain	336.600	5.400	24.930	1.320
L. sativus Hungery	334.560	2.300	20.980	0.860
Mean ± SE	348.16 ± 21.81	3.50 ± 1.66	23.10 ±1.99	1.24 ± 0.34
Min	334.56	2.30	20.98	0.86
Max	373.32	5.40	24.93	1.99
C.V	0.060	0.474	0.086	0.274
L. sativus Tunisia	320.280	3.254	31.530	0.560
Mean ± SE	320.28	3.25	31.53	0.56
Min	320.28	3.25	31.53	0.56
Max	320.28	3.25	31.53	0.56
C.V	0.00	0.00	0.00	0.00

continued overleaf / nadaljevanje na naslednji strani

Sample	TPC, mg g–1	Ash content, %	Wt 100 seed, g	ODAP, %
L. sativus Pakistan	352.920	8.900	5.750	1.37
L. aphacce GRC	348.840	5.600	1.801	0.35
L. latifolius Netherland	373.320	10.800	3.589	0.51
L. annus Syria	373.320	7.200	3.224	0.39
L. hirsutus Tunisia	416.380	6.200	2.786	0.609
L. hirsutus USA	410.040	8.100	2.611	0.590
L. mamoratus Syria	401.880	7.800	1.468	0.350
L. inconspicuos Turky	244.800	3.700	2.730	0.19
Mean ± SE	365.19 ± 54.80	7.29 ± 2.16	2.99 ± 1.31	0.54 ± 0.36
Min	244.80	3.70	1.47	0.19
Max	416.38	10.80	5.75	1.37
C.V	0.150	0.296	0.438	0.666
L. sativus Sudan	314.160	4.400	11.150	3.260
L. sativus Iran	332.520	3.100	9.460	1.640
L. sativus Turky	367.200	4.600	11.507	1.280
L. ochrus Cyprus	342.720	2.800	10.337	1.960
L. ochrus India	330.480	2.700	10.995	1.670
L. ochrus Iran	367.200	2.800	11.738	1.28
L.ciceria Syria	361.080	3.300	8.915	0.500
L. ciceria Turky	389.640	2.780	9.324	1.020
L. ciceria Cyprus	263.160	1.700	7.063	0.800
L. ciceria Norway	226.440	3.340	8.420	0.79
L. pseudocicer Turky	244.800	1.400	7.103	0.502
L. blepharicar Syria	295.880	4.100	8.287	0.609
Mean ± SE	319.61 ± 52.25	3.09 ± 0.97	9.52 ±1.63	1.28 ± 0.79
Min	226.44	1.40	7.06	0.50
Max	389.64	4.60	11.74	3.26
C.V	0.163	0.255	0.171	0.617

Table 3. Principal component analysis in 66 accessions of *Lathyrus* spp., Eigen-values and percent of variation accounted by the first two principal components (PRIN1 and PRIN2)

Preglednica 3. Analiza glavnih component 66 akcesij *Lathyrus* spp., Eigen-vrednosti in odstotek variabilnosti, ki ga pojasnjujeta prvi komponenti (PRIN1 in PRIN2)

Trait	PRIN1	PRIN2
Protein content	0.731	–0.305
Ash content	0.632	0.289
100 Seeds Weight	–0.309	0.861
ODAP content	0.727	0.421
Eigen – value	1.557	1.095
Variation, %	38.925	27.370
Variation accumulated, %		66.295

Table 3 gives the results of principal component analysis for the studied quality traits. The first two principal components account for 66.295% of the total variance of all traits, indicating a high degree of correlation among the characters for the accessions analyzed. Separate percentages of variation attributable to the first two components by decreasing order were 38.925 and 27.370%. By examining the eigenvectors of individual components, indications may be obtained about their levels of association with the original traits. Protein, ash contents and ODAP showed higher coefficients in the first component (PRIN1), while 100 seeds weight was a primary source of variation with the largest coefficient (0.861) in second principal component

(PRIN2). According to these results, the first two components in the principal component analysis were only considered.

Cluster analysis based on coefficient of variances derived by K-means clustering gave eight clusters (Figure 1), accounting for a 77% share of original variation. Clusters 8, 4, 6 were most similar and also 5, 3, 7 were most similar. Cluster 2 was the most distant and consequently, the least similar one and cluster 1 stands in an intermediate position between cluster 2 and other clusters. 18 accessions were grouped in the largest cluster (cluster 3), while cluster 1, 2, 4, 5, 6, 7 and 8 include 13, 1, 10, 3, 1, 8 and 12 accessions, respectively.

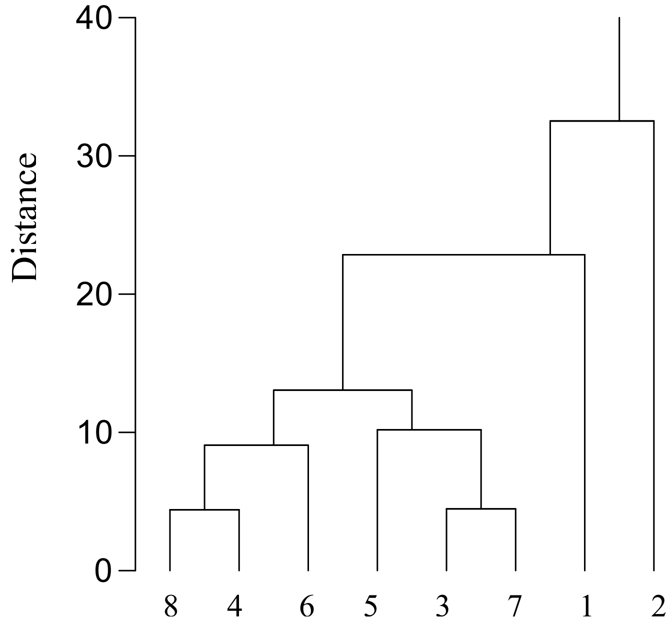

Figure 1. Dendrogram from cluster analysis of coefficient of variances derived from K-means clustering for four quality traits (total protein and ash contents, 100 seeds weight and ODAP percent) of 66 accessions of *Lathyrus* spp.

Slika 1. Dendrogram klastrske analize koeficientov variabilnosti, ki smo jih dobili iz povprečij za štiri lastnosti kakovosti (vsebnost celokupnih beljakovin in pepela, masa 100 semen in odstotek ODAP) 66 akcesij *Lathyrus* spp.

The means of the total proteins, ash content, 100 seeds weight and ODAP percent for each cluster were shown in Table 3. In this table, clusters 6 and 7, grouped the accessions with lower ODAP values and cluster 6 included the accession, which had the highest 100 seeds weight. The total protein contents varied between 395.83 mg g^{-1} in cluster 1 and 319.61 mg g^{-1} in cluster 8.

Regression analysis between ODAP and each of total protein content – TPC, ash content and 100 seeds weight for the studied accessions gave positive non significant correlation; R^2 equals 0.1381, 0.0139 and 0.0029 respectively. The results of variance analysis performed for individual trait showed that differences among accessions were statistically significant for all traits.

Table 4. Mean values of TPC, ash content %, 100 seeds wt and ODAP% for each of the eight groups

Preglednica 4. Srednje vrednosti vsebnosti celokupnih proteinov, odstotek pepela, masa 100 semen in odstotek ODAP za vsako od osmih skupin

Sample	TPC, mg g^{-1}	Ash content, %	Wt 100 seed, g	ODAP, %
1	395.83	3.71	2.78	1.34
2	391.68	7.400	3.427	2.070
3	358.14	4.24	7.49	0.85
4	327.01	3.81	14.17	0.94
5	348.16	3.50	23.10	1.24
6	320.28	3.25	31.53	0.56
7	365.19	7.29	2.99	0.54
8	319.61	3.09	9.52	1.28

DISCUSSION

Among the accessions analyzed none was classified as lacking the neurotoxin. In general the lowest β-ODAP levels (< 0.2%) were recorded in seeds of *L. inconspicuous* from Turkey, the highest value (> 6%) was recorded in seeds of *L. sylvestris* collected from USA. *L. sativus* accessions showed neurotoxin levels varying between 0.33% and 3.26. Our results indicated a high variability of level of β-ODAP at interspecific and intraspecific levels. Such variability of the level of β-ODAP in seeds of *L. sativus* accessions was in agreement with similar previous report by Polignano *et al*. (2005) who reported that the percent of β-ODAP varied from 0.24% to 0.64%. The content of β-ODAP in the examined accessions ranged between 0.19–6.23 percent. Although the extreme values of variation was similar to the data reported by Polignano *et al.* (2005), it was higher than the levels recorded by Urga *et al.* (2005), Yigzaw *et al.* (2001) and Urga *et al.* (1995). The variation in β-ODAP content between the different sets of data might be attributed to the method of analysis used (Tavoletti et al., 2005), environmental conditions and genetic factors (Dahiya, 1986; Barat *et al.,* 1989; Siddique *et al.,* 1996). The β-ODAP was determined by colorimetric and capillary electrophoresis analyses. A high positive correlation between the two methods was found (r= 0.83), but the colorimetric values showed, on average, significant 14% lower ODAP values (Tavoletti *et al.*, 2005). In this piece of work, the colorimetric method was used to determine β-ODAP in the accessions of *Lathyrus* spp.

Regression analysis between ODAP and each of total protein content, ash content and 100 seeds weight for our materials was non significant, indicating that these traits did not have any significant correlation. The same conclusion was deduced by Roy and Rao (1978) in their work on twenty nine varieties of *L. sativus* seeds. However, Urga *et al.* (2005) found a significant positive correlation between β-ODAP and crude protein content. The discrepancy between the data of Urga *et al*. (2005) and ours was attributed to the difference between crude protein and total protein. Whereas the crude protein was estimated from measuring the total nitrogen content, coming from both protein and non-protein nitrogen sources, the total protein reflects on the nitrogen associated with protein not include the nitrogen from non-protein source.

The results of variance analysis performed for individual trait showed that differences among accessions were significant for all traits. ODAP showed the highest coefficient of variation (CV) (96.5%), on the contrary protein content showed lowest coefficient variation (15%). Intermediate values of variation were shown by 100-seeds weight (71.9%) and ash content (46.6%). The highest variability in β-ODAP and 100-seeds weight were attributed to the different taxa used in this study and to the different geographical regions and habitats the taxa collected from.

It was reported that *L. sativus* and *L. cicera* included the most interesting entries concerning low ODAP level (Granati *et al.,* 2001). Our results showed that this finding cannot stand up. It was found that accessions belonging to *L. inconspicuous, L. aphaca, L. hierosolymitan; L. tingitanus* had an amount of β-ODAP less than that found in *L. saivus* and *L. cicera.*

The variation in β-ODAP between the accessions of the same taxa was due to the variation in water stress. It was found that the severely stressed plants had significantly higher β-ODAP concentration than unstressed plants (Swarup and Lai, 1993), it was also found that ODAP concentration is affected by phenology as well as by both genetic control and environmental conditions (Lambein *et al.,* 1990). Our alternative explanation is that the lower concentration found in good environment is the result of toxin dilution, suggesting a restricted "pool" of toxin is distributed over a large number and weight of seeds.

It was suggested that β-ODAP accumulation in grass pea might be related to the level of total free nitrogenous compounds and that nitrogen and phosphate may be crucial nutrient factors influencing β-ODAP content under field conditions (Jiao *et al*., 2006). Thus the application of appropriate nitrogen and phosphorus fertilizers to the soil may decrease the content of β-ODAP in the seeds and leaves of grass pea.

Protein content in the seeds of *Lathyrus spp* ranged from 22.6 to 49.3 with mean value 35.4%. The highest content was recorded in *L. sylvestris* from USA (49.36%) and the lowest in *L. cicera* from Norway (22.6). As far as known, majority of study on total protein content in genus *Lathyrus* was directed to *L. sativus*. That was the interpretation for the contradictory between our results and that reported by Hove and King (1978), Shobhana *et al.*, (1976), Urga *et al.*, (2005), Granati *et al.*,(2001), Roy and Rao (1978) who reported a protein content ranked between 23 and 31% with mean value 29.7%.

The ash content assessed in 66 accessions in *Lathyrus spp* showed variation ranging from 1.2 to 8.6%. One hundred seed weight was more variable and ranged from 1.35 to 31.5 g. The largest was found in accessions of *L. sativus*. This data was in agreement with the result of Granati *et al*. (2001) and Della and Polignano (2002). Since *L. sativus* has grown in a good environment as a cultivated plant and there was a negative relationship between seed yield components and stresses, the highest 100-seeds weight of *L. sativus* can be justified. However, there was no positive correlation between 100-seed and β-ODAP within the accessions of the other taxa. In the light of the suggestion that seed weight was negatively correlated with stresses and β-ODAP content (Urga *et al*., 2005), and the contradictory trend found in our results, we inferred that the relationship between seed weight and β-ODAP couldn't be simplified in stresses only. It might be influenced by both environmental and genetic factors, but the mechanism by which the environment acts is not clear.

Multivariate analysis (principal co-ordinate analysis), showed a sort of association between protein, ash content and β-ODAP. This association can be interpreted in terms of the role of (1) mineral (Zinc, Calcium, Phosphorus and Molybdenum) which is the main component of the ash in stimulating β-ODAP accumulation in the seeds and (2) the crucial role of free nitrogenous compounds in influencing β-ODAP content under the field. It was found that the decrease in free nitrogenous compounds in the developing seed was accompanied by a rapid accumulation of protein (Jiao *et al*., 2006), and that the free nitrogenous compounds such as glutamine and serine as well as nucleotide nitrogen, all significantly enhanced the accumulation of β-ODAP in young seedling (Lambein *et al*., 2007).

Based on cluster of C.V values for each accession, eight groups are identified; accounting for a 77% share of original variation, the most promising accessions was *L. sativus* accessions collected from Tunisia. This accession grouped in cluster 6, being good accession for grain quality, due to their relatively low ODAP level and high protein content.

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