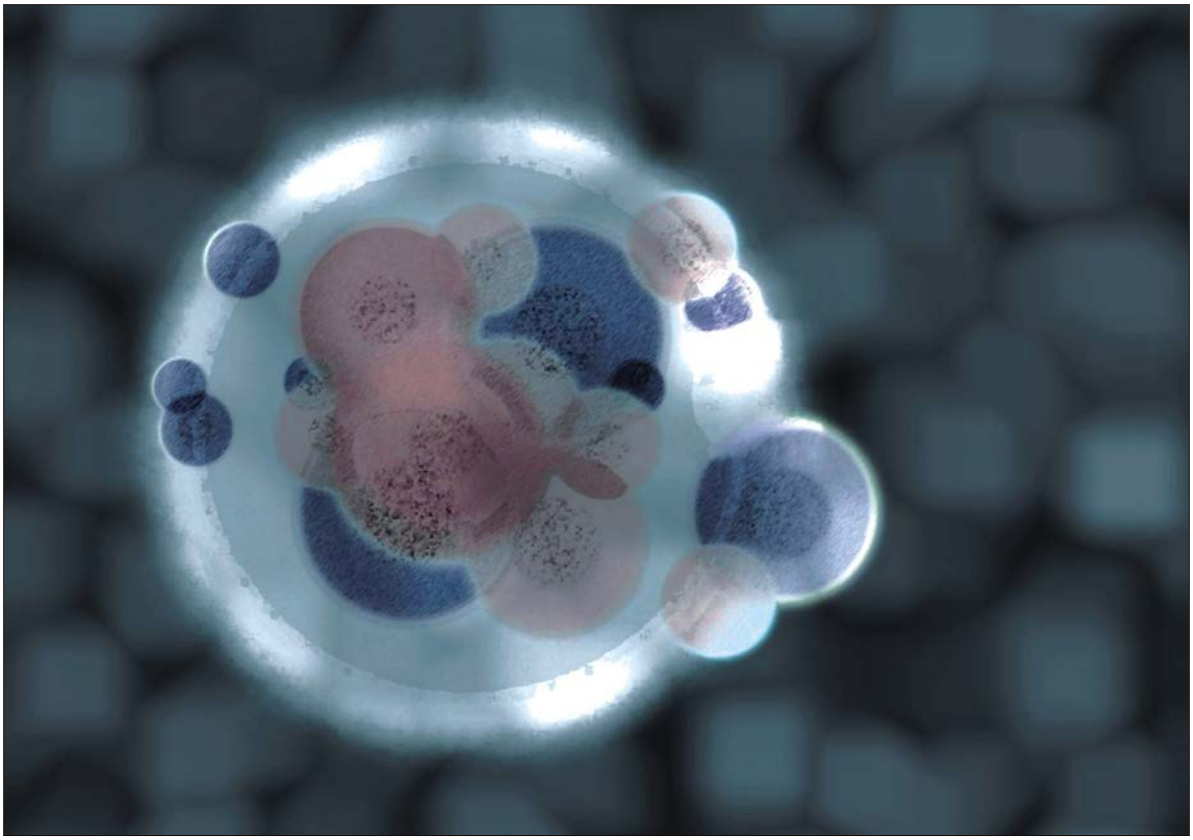

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Evaluation of *Lathyrus* spp. Germplasm for Quality Traits

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Abstract: Sixty six accessions representing eighteen species of the genus *Lathyrus* collected from different geographic regions were evaluated for variations of quality traits (protein, ashes, 100 seeds weight and ODAP). High variability of ODAP levels were exhibited at both inter-specific and intra-specific levels, which 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 CV values for each accession identified the sixty six accessions into eight groups. The most promising accession for breeding programs was *L. sativus* from Tunisia, having good grain quality due to relatively low ODAP level and high protein content. The variation of protein content, ash content and 100 seeds weight was also discussed.

Key words: *Lathyrus* spp., breading, quality trait, ODAP, genetic variation

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 Asian 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 marginal lands in Syria, Lebanon, Egypt, Libya, Algeria, Morocco, France and Spain, the Mediterranean basin.

The most important traits of *Lathyrus* consist of drought tolerance, resistance of stored grains to pests, adaptability to nearly all types of soils as well as to adverse climatic conditions and low input environment (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 limited uses due to the presence of a neurotoxic compound, which 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 (Shah, 1939). The consumption of the grains of *L. sativus* is limited due to the presence of the neurotoxic compound 3-(-N-oxayl)-L-2,3 diaminopropoinc acid (ODAP). The other forms of the toxic substance in *L. sativus* are (Beta-N-oxalyl-alpha-beta diamino Propionic acid) and BOAA (Beta-N-oxalyl amino-L-Alanine) (Smartt *et al*., 1994; Williams *et al*., 1994). The neurotoxic compound was also detected in the genus *Vicia*, which have similar effects on experimental animals (Duke, 1981; Williams *et al*., 1994).

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 (Hunbury *et al*., 2000). Campbell and Briggs (1987) reported *L. sativus* variety (Var. 8246) having a low ODAP content of 0.0259-0.0401% (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 neurotoxin presence, *Lathyrus* product has been banned in many countries. However, due to the importance of this crop in developing countries, these countries have established of breeding programmes mainly focused on getting a genotype with high seed yield 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, 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 Khan *et al*., 2001; Ben Brahim *et al*., 2001). To follow such way, enough information about genetic diversity and genetic resources of *Lathyrus* germplasm

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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 advocated to ascertain the genetic variability present in *Lathyrus* germ plasm of different origin (Infantino *et al*., 1994; Granati *et al*., 2000; Alba *et al*., 2001; Polignano *et al*., 2001).

In this study, we aimed at evaluating quality traits (protein, ashes, 100 seed weight and ODAP) in 66 accessions belong to 18 species and have a world wide distribution. The selection for recovering the accessions with superior quality traits introduces a valuable genetic material for local or national breeding programmes.

MATERIALS AND METHODS

This study was conducted at Botany Department, Faculty of Science, Tanta University at 2005-2006. Seeds of *Lathyrus* spp. were collected represented all environments in all over the world which came from International Center for Agricultural Research in the Dry Areas (ICARDA) and Poston; the other seeds of *Pisum* were obtained from Egypt and *Vicia faba* var. Giza 843 were obtained from the Agricultural research center, El-Dokki, Giza, Egypt.

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 h at −4°C. After that time, the extracts were centrifuged for 10 min at 1000 g. Quantitative estimation of total proteins was made according to Bradford (1976). Three replicas were made for each sample.

100 seeds weights: One hundred seed weight in each sample and notes it; the different in weight present in each sample determine the genetic diversity.

Ash content: The ash content was estimated after combustion of the oven dried sample at 500°C for 1 h. The ash content is a measure of the total mineral content.

ODAP content: Neurotoxin level was analyzed following a modified Rao (1978) procedure. 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 to 0.33 mL of KOH 3N. The sample (4 replica/genotype) was kept in a boiling water bath for 30 min (alkaline hydrolysis to convert from ODAP to DAP (Diamino Pripionic Acid) which can be determined) and then brought to 1 mL with water.

To detect ODAP, OPT reagent was used, it was composed of 100 mg of OPT (Orthophthaldehyde), 1 mL ethanol 95%, 0.2 mL of mercaptoethanol and 99 mL of potassium tetra borate buffer (0.05 M in distilled water, pH 9.9). This reagent was freshly made and used for long as 3 days. 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.

$$A = C \varepsilon L$$

Where:
A = Absorbance
C = Concentration
ε = Extinction co-efficient absorbitinty is constant and
L = 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

Regression analysis between ODAP and each of total protein content, 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 (Fig. 1A-C).

The results of variance analysis performed for individual trait showed that differences among accessions are statistically significant for all traits. Protein content ranged from 22.6-49.3% with mean value of 35.4%. Extreme ODAP levels are 0.19 and 6.2% showing mean value of 1.05%. One hundred seed 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). 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 (Table 2). Separate percentages of variation attributable to the first two components by decreasing order are 38.925 and 27.370%. By examining the eigenvectors of individual components, indications may be obtained about their levels of association with

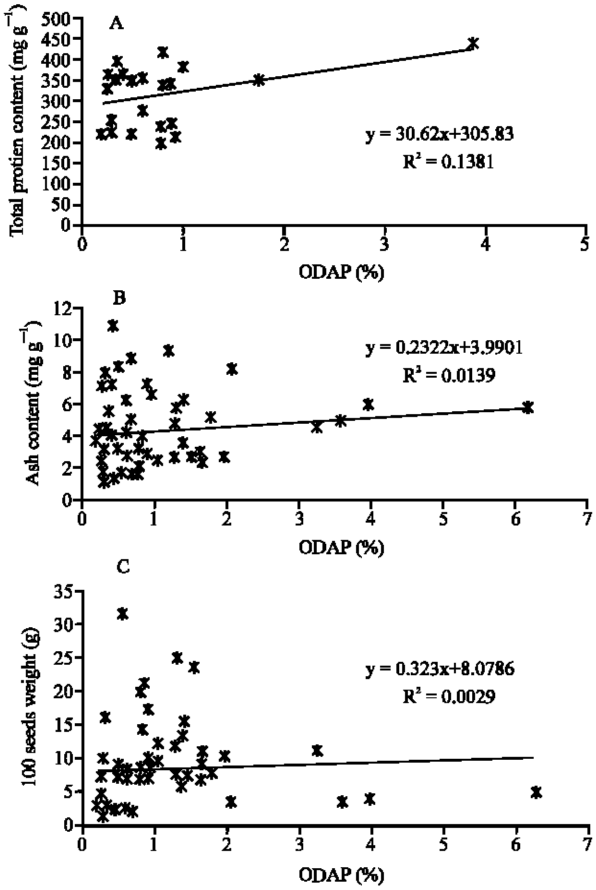

Fig. 1: Regression analysis between ODAP and each of TPC (A), ash content (B) and 100 seeds weight (C)

Table 1: Mean values, Standard Error (SE), ranges, coefficients of variation (CV) observed in 66 accessions belonging to 18 species of *Lathyrus* spp.

Traits	Mean±SE	Min	Max	CV
Protein content (mg g^{-1} dry wt.)	354.166±53.091	226.440	493.6800	0.150
Ash content (%)	5.147±8.089	1.200	8.6000	1.571
100 seeds weight (g)	8.472±6.019	1.354	31.5300	0.710
ODAP content (%)	1.051±1.036	0.190	6.2300	0.985

Table 2: Principal component (PRIN) analysis in 66 accessions of *Lathyrus* spp., Eigen-values and percent of variation accounted by the first two principal components

Traits	PRIN 1	PRIN 2
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 (%)	66.295	

the original traits. Protein, ash contents and ODAP show higher coefficients in the first component (PRIN 1), while the second component (PRIN 2) shows significant shares

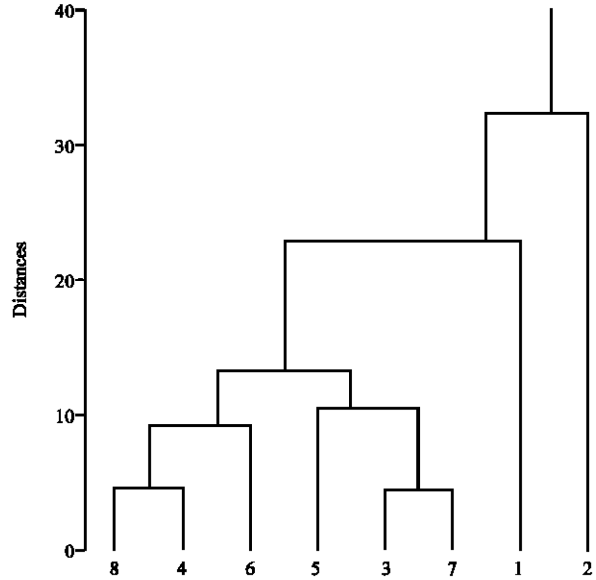

Fig. 2: Dendrogram from cluster analysis of 66 accessions using uncorrelated traits (PC coefficients)

of variation to associate with the 100 seeds weight. According to these results, the first two components in the cluster analysis were considered.

Eight groups are identified, accounting for a 77% share of original variation which corresponds to genetic variation which corresponds to 33% distance. Group 8, 4, 6 are most similar and also 5, 3, 7 are most similar (Fig. 2). Group 2 is the most distant and consequently, the least similar one and Group 1 stands in an intermediate position between group 2 and other groups. 18 accessions are grouped in the largest cluster (Group 3), while cluster 1, 2, 4, 5, 6, 7 and 8 include 13, 1, 10, 3, 1, 8 and 12 accessions, respectively. Cluster memberships, means, minimum and maximum values for each cluster are shown in Table 3. Each cluster includes different proportions of populations, accessions belong to different species and from different countries, except cluster 2, 5 and 6 are homogenous in terms of the species they belong to.

In particular cluster 1 includes accessions belong to *L. aphacce*, *L. sphaerius*, *L. hirsutus*, *L. sylvestris*, *L. hierosolymitan*, *L. gorgoni* and *L. inconspicuos* and shows the highest mean value for the total protein content. Clusters 2 and 6, the smallest clusters grouped with one accession each, include *L. sylvestris* Germany and *L. sativus* Tunisia, respectively. It is interesting to note that cluster 2% 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

Table 3: K-means clustering based on total protein (TPC) and ash contents, 100 seeds weight (wt.) and ODAP%, cluster memberships, Cluster memberships, cluster mean, coefficient of variation, minimum and maximum values

Sample	TPC	Ash content (%)	100 seed wt. (g)	ODAP (%)
L. aphacce Syria	364.100	3.300	3.552	0.300
L. aphacce Iran	416.160	4.500	1.525	0.240
L. sphaerius Syria	369.240	1.200	2.242	0.320
L. sphaerius Turkey	363.120	1.900	1.767	0.350
L. hirsutus Egypt	399.840	4.300	2.900	0.320
L. hirsutus France	471.240	4.800	2.151	0.685
L. hirsutus Turkey	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 Kazakhistan	440.640	5.500	3.975	3.970
L. hierosolymitan Turkey	371.280	2.400	4.014	0.259
L. gorgoni Turkey	367.200	4.500	2.893	0.335
L. incouspicuos Yughoslavia	252.960	1.800	1.354	0.300
Mean±SE	395.83±	3.71±	2.78±	1.34±
	60.130	1.450	0.970	1.950
Min	252.960	1.200	1.350	0.240
Max	493.680	5.500	4.120	6.230
CV	0.152	0.390	0.348	1.455
L. sylvestris Germany	391.680	7.400	3.427	2.070
Mean±SE	391.680	7.400	3.430	2.070
Min	391.680	7.400	3.430	2.070
Max	391.680	7.400	3.430	2.070
CV	0.000	0.000	0.000	0.000
L. sativus Ethopia	291.720	5.800	7.720	1.400
L. sativus Bangladesh	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	1.310
L. clymenum Portugal	361.080	4.080	7.632	0.820
L. clymenum Turkey	363.120	3.100	7.307	0.330
L. latifolius Netherlands	401.880	3.600	5.845	0.300
L. tingitanus Portugal	373.320	7.010	9.443	0.280
L. annus Turkey	395.800	3.060	6.479	1.640
L. sylvestris Moraco	422.280	8.600	8.452	0.820
L. hierosolymitan Plastine	344.760	2.200	7.036	0.270
L. gorgoni Jordan	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 Australia	361.080	1.700	7.063	0.600
L. mamoratus Turkey	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	0.940
Mean±SE	358.14±	4.24±	7.49±	0.85±
	39.290	1.840	0.890	0.490
Min	242.760	1.700	5.840	0.270
Max	422.280	8.600	9.440	1.790
CV	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 Afghanistan	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 Canada	328.440	6.900	17.240	0.914
L. ochrus Portugal	336.600	3.600	15.426	1.400
L. pseudocicer Jordan	269.280	3.040	9.974	0.910
L. blepharicar Turkey	275.400	3.300	9.872	0.304
Mean±SE	327.01±	3.81±	14.17±	0.94±
	38.640	1.180	3.150	0.400
Min	269.280	2.600	9.870	0.300
Max	408.000	6.900	19.880	1.410
CV	0.120	0.310	0.222	0.425

Table 3: Continued

Sample	TPC	Ash content (%)	100 seed wt. (g)	ODAP (%)
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±	3.50±	23.10±	1.24±
	21.810	1.660	1.990	0.340
Min	334.560	2.300	20.980	0.860
Max	373.320	5.400	24.930	1.990
CV	0.060	0.474	0.086	0.274
L. sativus Tunisia	320.280	3.254	31.530	0.560
Mean±SE	320.280	3.250	31.530	0.560
Min	320.280	3.250	31.530	0.560
Max	320.280	3.250	31.530	0.560
CV	0.000	0.000	0.000	0.000
L. sativus Pakistan	352.920	8.900	5.750	1.370
L. aphacce GRC	348.840	5.600	1.801	0.350
L. latifolius Netherlands	373.320	10.800	3.589	0.510
L. annus Syria	373.320	7.200	3.224	0.390
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. incouspicuos Turkey	244.800	3.700	2.730	0.190
Mean±SE	365.19±	7.29±	2.99±	0.54±
	54.800	2.160	1.310	0.360
Min	244.800	3.700	1.470	0.190
Max	416.380	10.800	5.750	1.370
CV	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 Turkey	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	1.280
L. ciceria Syria	361.080	3.300	8.915	0.500
L. ciceria Turkey	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	0.790
L. pseudocicer Turkey	244.800	1.400	7.103	0.502
L. blepharicar Syria	295.880	4.100	8.287	0.609
Mean±SE	319.61±	3.09±	9.52±	1.28±
	52.250	0.970	1.630	0.790
Min	226.440	1.400	7.060	0.500
Max	389.640	4.600	11.740	3.260
CV	0.163	0.255	0.171	0.617

one, shows the lowest mean value for ODAP (0.54) and intermediate values for the other traits (Table 4). The low ODAP accessions are represented by *L. inconspicuos* Turkey. The relations between the values of 100 seeds weight, total protein and ash contents and ODAP percent for the accessions of each cluster showed opposite relation between total protein content and other traits which exhibited parallel relation with each other (Fig. 3).

Results of principal component analysis were combined with the results of cluster analysis in a two dimensional plot of the first two component scores (Table 2). Each component score is a linear combination of the traits, such that the first maximum amount of variance is shown on the first principal component, second maximum amount on the second component.

Table 4: Mean values of total protein content (TPC), ash content %, 100 seeds weight (w.t) and ODAP% for each of the eight groups

Sample	TPC	Ash content (%)	100 seed wt. (g)	ODAP (%)
1	395.83	3.710	2.780	1.340
2	391.68	7.400	3.427	2.070
3	358.14	4.240	7.490	0.850
4	327.01	3.810	14.170	0.940
5	348.16	3.500	23.100	1.240
6	320.28	3.250	31.530	0.560
7	365.19	7.290	2.990	0.540
8	319.61	3.090	9.520	1.280

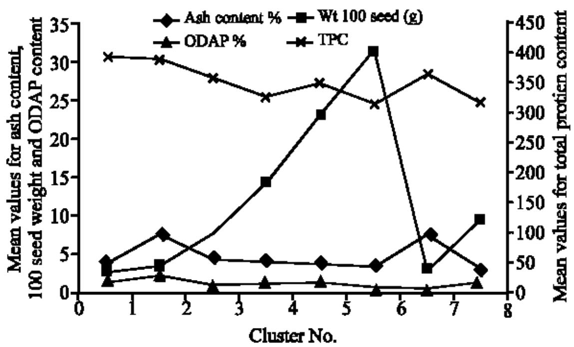

Fig. 3: The relationship between mean values of total protein content (TPC), ash content %, 100 seeds wt and ODAP % for each of the eight groups

Two-dimensional distances between accessions (Table 2) reflects a summary of differences between accessions based on four traits measured to the extent that the first two principal components are effective in capturing the combined variance of four traits. Compared with other clusters, that are tightly grouped, cluster 2 was relatively distant (Fig. 2). It is to underline that 100 seeds weight is a primary source of variation with the largest coefficient (0.861) in second principal component, axis (Table 2). Clusters 6 and 7, which are differentiated by K-means clustering (Table 3) include accessions with lower ODAP values.

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. Present results indicate 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 is in agreement with earlier results reported by Polignano *et al.* (2005) who reported that the percent of β-ODAP varied from 0.24-0.64%. The content of β-ODAP in the examined accessions ranges between 0.19-6.23%. Although the extreme values of variation is 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 is 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 study, 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 study on twenty nine varieties of *L. sativus* seeds. However, Urga *et al.* (2005) found a significant positive correlation between β-ODAP and curde protein content. The discrepancy between the data of Urga *et al.* (2005) and ours is the difference between crude protein and total protein. Whereas the crude protein is 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 are 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* include the most interesting entries concerning low ODAP level (Granati *et al.*, 2001). Present results showed that this finding can not stand up. It was found that accessions belonging to *L. inconspicuous*, *L. aphaca*, *L. hierosolymitan*, *L. tingitanus* have 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* is directed to *L. sativus*. That is the interpretation for the contradictory between present results and that reported by Hove and King (1978), Shobhana *et al.* (1976), Urga *et al.* (2005), Granati *et al.* (2001) and Roy and Roa (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 1.2-8.6%. One hundred seed weight was more variable and ranged from 1.35-31.5 g. The largest was found in accessions of *L. sativus*. This data is in agreement with the result of Granati *et al.* (2001) and Bisignano *et al.* (2002). Since *L. sativus* grown in a good environment as a cultivated plant and there is a negative relationship between seed yield components and stresses, the highest 100 seeds weight of *L. sativus* can be justified. However, there is 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 is negatively correlated with stresses and β-ODAP content (Urga *et al.*, 2005) and the contradictory trend found in present results, we inferred that the relationship between seed weight and β-ODAP can not 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 a 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 CV values for each accession, eight groups are identified; accounting for a 77% share of original variation, the most promising accessions is *L. sativus* accessions collected from Tunisia. This accession grouped in group 6, being good accession for grain quality, due to their relatively low ODAP level and high protein content.

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