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Full Length Research Paper

Some quality traits and neurotoxin β-*N*-oxalyl-L-α,β-diaminopropionic acid (β-ODAP) contents of *Lathyrus* sp. cultivated in Turkey

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In this study, 52 landraces accessions belonging to the species of *Lathyrus sativus* and *Lathyrus clymenum* collected from different regions of Turkey and one released variety of *Lathyrus sativus* were evaluated for some quality traits like seed coat color, 1000 seed weight, crude protein and neurotoxin β-*N*-oxalyl-L-α,β-diaminopropionic acid (β-ODAP) content. Among the investigated landraces, high variation was determined for all the investigated traits, which was attributed to both genetic as well as environmental factors. Protein content was as between 24.07 to 30.90% and β-ODAP content was as 1.35 to 3.86 mg g^{-1} for seed. Many landraces with low β-ODAP content (< 2.00 mg g^{-1}) and high protein content seem to be promising material for *Lathyrus* breeding. Furthermore, many investigated landraces showed lower β-ODAP and higher protein contents compared with the released variety. Also, the local consumption of *L. sativus* landraces as a food or feed was determined during the collection process.

Key words: *Lathyrus*, landrace, protein, β-ODAP, Turkey.

INTRODUCTION

Lathyrus sativus L. (grass pea, in Turkish "murdumuk, culban, fasil, feslek") is a traditional crop use for both animal consumption as forage and grain and for human consumption as a pulse. *L. sativus* has many advantages including drought and flooding tolerance, disease resistance, high yield potential and favorable nutritional composition of seeds. It can easily grow in different soil types and at high altitudes (Tivari and Campbell, 1996a, b). *L. sativus* grows with as little as 250 mm of annual precipitation and it is typically the last surviving plant in drought times (Tekele-Haimanot et al., 1990; White et al., 2002).

Besides the obvious advantages, *L. sativus* seeds contain a major anti-nutritional compound namely β-ODAP (Zhao et al., 1999; Wang et al., 2000). Heavy and prolonged (3 to 4 month) consumption of *L. sativus* seeds can cause a disease known as 'lathyrism' or 'neuro-lathyrism', a disease causing paralysis of the limbs (Spencer et al., 1986; Yan et al., 2006).

Until recently, relatively little effort has been made towards the improvement of this hardy pulse crop due to its toxicity. To counter the effects of climate change on agricultural productivity, *L. sativus* is gaining interest in southern Australian Mediterranean-type environments (Hanbury et al., 1999), North America (Campbell et al., 1994) and Southern Europe (Crino et al., 2004; Tavoletti et al., 2005; Polignano et al., 2009). Also, many studies have been carried out to bring grass pea in areas under monoculture cereal cultivation in Europe (Vaz Patto et al., 2006).

In order to reintroduce *L. sativus* in crop rotations under marginal environments, breeding programs are in progress worldwide to improve genotypes combining high yield, high protein content and low or zeroneurotoxin content (ODAP) (Bozzini, 1997; Siddique et al., 1998; Hanbury et al., 2000).

Lathyrus cultivation was not well documented in

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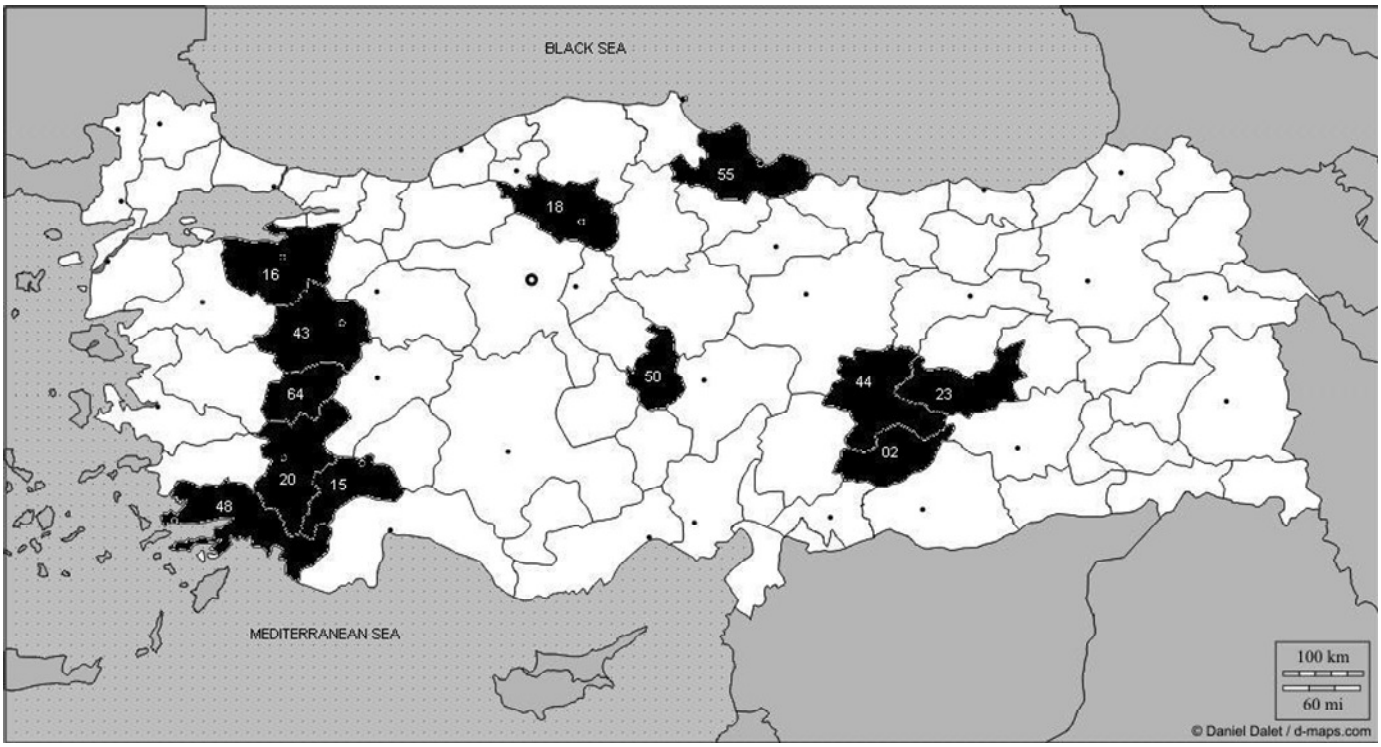

Figure 1. Distribution of the collection area in Turkey. 02, Adiyaman; 15, Burdur; 16, Bursa; 18, Cankiri; 20, Denizli; 23, Elazig; 43, Kutahya, 44, Malatya; 48, Mugla; 50, Nevsehir; 55, Samsun; 64, Usak.

Turkey. However, some authors reported that, *L. sativus*, *Lathyrus cicera*, *Lathyrus clymenum* and *Lathyrus ochrus* were cultivated in Turkey (Genc and Sahin, 2001; Cetin, 2006) and *L. sativus* was widely cultivated in the country in the past (Davis, 1970). In addition, *Lathyrus hirsutus* had been cultivated in the east and inner parts of Turkey until 1960s (Tosun, 1974). *Lathyrus* cultivation dramatically decreased from the 1960s. Today, based on actual data, only *L. sativus* is cultivated in 18.000 ha for the seed in Turkey (TUIK, 2008). This crop is mainly used as stock-feed; however, it is rarely used for human cunsumption in some parts of the country (Basaran et al., 2010).

There is only one released variety of *L. sativus*, 'Gürbüz 2001' in Turkey. This means, approximately all the cultivated genotypes are landraces; hence, they are promising materials for breeding programs. Most of these landraces were not previously screened. However, Karadag et al. (2010) indicated that, it had a high variability in β-ODAP levels in winter and spring-sown grasspea lines. On the other hand, interestingly, there is no lathyrism case documented for both humans and animals in Turkey which may result from landraces with low β-ODAP content. So, the screening of these landraces regarding their β-ODAP content is very important.

For this purpose, expeditions were carried out to collect *Lathyrus* landraces cultivated in Turkey and to evaluate their quality traits including the protein and β-ODAP contents. Also, this study provided comprehensive and additional information about the general status of *Lathyrus* cultivation in Turkey.

MATERIALS AND METHODS

In this study, a total of 52 local landrace accessions of *Lathyrus* (51 *L. sativus* and 1 *L. clymenum*) collected from Turkey were examined. To collect *Lathyrus* accessions, initially 12 cities in which *Lathyrus* are cultivated were determined based on data of the Turkish Statistical Institute (TUIK, 2007) and Ministry of Agriculture records. The collection program was carried out in 2007. The collection area is given in Figure 1.

Lathyrus accessions were taken from different farmers or local seed markets and their name and altitude of collection cites were recorded. Three landraces of the collected *L. sativus* genotypes are commercial material. In addition, the released variety 'Gürbüz-2001' was obtained from Turkish Central Research Institutes for Field Crops. Seed coat color, 1000 seed weight, protein and β-ODAP contents were determined for each sample.

Seed coat color was determined by visual observation. Total nitrogen was determined by the Kjeldahl method and crude protein content was estimated using a conversion factor of 6.25 (Kacar, 1972). All the data were presented as mean, mimimum, maximum and standard deviation determined by using SPSS 13.0 statistical package program.

Determination of β-ODAP

β-ODAP was determined using capillary zone electrophoresis (CZE) (Zhao et al., 1999) with some modifications. CZE was carried out using Agilent HP^{3D} and UV detection at 195 nm. The

capillar was 55 cm (44 cm effective length) x 50 µm. The analyses were performed at a constant voltage of 20 kV at 20°C in an electrolyte of 75 mM (H_3BO_3) buffer at pH 7.5. The required pH of the buffer was adjusted by adding NaOH. All chemicals were of analytical-reagent grade. The reference standard of β-ODAP was obtained from Dr. S.L.N.Rao (Lathyrus Technologies, Hyderabad, India).

Sample extraction

0.5 g powder of *L. sativus* seeds was soaked in 50 ml ethanol-water (30:70, v/v) solution and was shaken for 2 h (in ice). After centrifugation (3500 rpm at 15 min), the upper clear solution was filtered with 0.45 µm filter paper. Then clear solution was diluted with ultra distilled water (1:1) and was injected directly into the CZE system for 40 s at 50 mbar.

RESULTS AND DISCUSSION

There was very limited information about the distribution and density of *Lathyrus* cultivation in previous years. But, according to the old farmer's statements, *Lathyrus* cultivation was more widespread in the past than now and a dramatically decrease begun in the 1960's. During the collection expedition, only landraces of *L. sativus* and *L. clymenum* species were collected. But *L. cicera*, *L. ochrus* and *L. hirsutus* were not encountered; their cultivation was reported by different authors (Tosun, 1974; Genc and Sahin, 2001; Cetin, 2006). The absence of *L. cicera* and *L. ochrus* among the encountered species in the study clearly indicated that, the cultivated *Lathyrus* materials had been exposed to genetic erosion in the last 50 years and a lot of genetic materials disappeared during the period.

The collection sites and local names of *Lathyrus* landraces throughout the country are presented in Table 1. Seed trade for both *L. sativus* and *L. clymenum* was very limited and also, there was only one released variety named 'Gürbüz-2001' in Turkey. So, farmers usually sow their own seed produced in previous years.

In general, *L. sativus* is cultivated at high altitudes (600 to 1600 m). The common name of *Lathyrus* species is "murdumuk" in Turkey. Moreover, *Lathyrus* is known by many local names such as "culban, coluk, fasil feslek" and *L. clymenum* is known as "kara murdumuk" (Table 1).

L. sativus is mainly cultivated for its seed and it is used as feed (Table 2). In some cities, however, its grain has been rarely consumed by humans as a soup, pilaf or snack (by mixing with chickpea). *L. clymenum* is cultivated only in Mugla city in small field for household consumption and its seed is used to make soup and it is added in the traditional food named "stuffed zucchini flowers".

According to farmers, *L. sativus* has higher seed yield than other legumes especially in dry condition and do not shatter its pods. So, farmers prefer to harvest the plant for seed production than for forage. For animal feeding, seeds are used by soaking in water or grinding and mixing with cereals in Turkey.

Seed coat colors amongst the landraces of *L. sativus* examined in the study were grey, brown, creamy and mostly mixture of these color. Creamy seed coat color was observed in few landraces in the mixture. However, landrace 5501 used only for human consumption had creamy seed coat color alone. The color variation in a single landrace can be related to genetic variability. So, besides inter population variation, intra population genetic variation was as high as possible, which is a desired feature in plant breeding. On the other hand, genotypes with light cream color seed had low neurotoxin content (Dahiya, 1976). It was reported that, seed coat color could be a useful visual character to select low toxic lines (Campbell, 1997). However, Kaul et al. (1986), did not find any relationship between seed coat color and toxic contents. In this study, certain relationship between seed color and β-ODAP contents was not determined. Many landraces with grey or brown seed coat color had low β-ODAP than creamy seed's landraces. Moreover, the 6406 numbered landraces, which had low toxicity, had grey and brown seed color (Table 2).

1000 seeds weight changed from 72.2 g in landrace numbered 2303 to 148.0 g in landrace numbered 5501 (Table 2) with mean 100.2 g (Figure 2). According to farmers' experience, they had not observed pod shattering in these landraces. *L. sativus* showed high variations (41 to 510 g) regarding 1000 seed weight (Joshi, 1997; Rybinski et al., 2008). Larger-seeded types are mostly found around the Mediterranean region, while small-seeded types are found in Indian subcontinent. In addition, small-seeded types have a tendency for pod shattering (Campbell, 1997). So, in general, the cultivated *L. sativus* landraces in Turkey showed similar features with the Mediterranean genotypes.

Crude protein (CP) contents of the landraces varied from 24.07 to 30.90% for *L. sativus* and it was 26.33% for *L. clymenum*. CP content in the released variety was 25.81% (Table 2.) and mean CP content was 28.23% for all the landraces (Figure 3). Variation in the seed CP contents can be attributed more to ecological factors other than genetic variation due to the ecological differences of the collection areas. But, *Lathyrus* is mostly cultured on poor or marginal lands with no fertilization in Turkey. So, these hard growing conditions determined the high CP ratios (28.23% as a mean) which indicated that many of the collected landraces may display genetic ability for high protein content. The seed CP ratios obtained in this study are similar to the results of other authors (Rotter et al., 1991; Rosa et al., 2000; Urga et al., 2005; Karadag et al., 2009; Karadag and Yavuz, 2010) who reported protein content for *L. sativus* seed between 21.49 and 31.98%. But our results were lower than the results of Abd El Zaher et al. (2007) who reported that, seed protein was between 29.17 and 37.33% in *L. sativus* and between 36.11 and 36.72% in *L. clymenum*.

Table 1. Collection sities and local name of accessions of *Lathyrus* sp.

Number	Accession**	City	Village/district	Local name	Altitude (m)
1	LS-0201	Adiyaman	Dardogan/ Merkez	Culban/ Coluk	825
2	LS-0202		Büklum/ Merkez	Culban/ Coluk	787
3	LS-1501	Burdur	Kizilkaya/ Bucak	Murdumuk	787
4	LS-1502		Harmanli/ Yesilova	Murdumuk	1093
5	LS-1503		Kizilkaya/ Bucak	Murdumuk	787
6	LS-1601	Bursa	Dogancılar/ Harmancık	Murdumuk	781
7	LS-1602		Dogancılar/ Harmancık	Murdumuk	781
8	LS-1603		Demirciler/ Harmancık	Murdumuk	719
9	LS-1604*		Commercial	Murdumuk	-
10	LS-1801	Cankiri	Yukaripelitozu/ Merkez	Murdumuk	768
11	LS-1802		Yukaripelitozu/ Merkez	Murdumuk	768
12	LS-1803		Elmaci / Eldivan	Murdumuk	957
13	LS-2001	Denizli	Uzunpinar/ Merkez	Murdumuk/Fasil	1148
14	LS-2002		Cabar/ Civril	Murdumuk/Fasil	1013
15	LS-2003		Ulukent/ Tavas	Murdumuk/Fasil	934
16	LS-2004		Balkica/ Tavas	Murdumuk/Fasil	1150
17	LS-2005		Eziler/ Guney	Murdumuk/Fasil	841
18	LS-2006		Baklancakirlar/ Cal	Murdumuk/Fasil	886
19	LS-2007		Alaaddin/ Acipayam	Murdumuk/Fasil	887
20	LS-2008		Karahoyukavsari/ Acipayam	Murdumuk/Fasil	900
21	LS-2301	Elazıg	Uzuntarla/ Merkez	Culban	995
22	LS-2302		Acipayam/ Merkez	Culban	987
23	LS-2303*		Commercial	Culban	-
24	LS-4301	Kutahya	-/ Domonic	Murdumuk	880
25	LS-4401	Malatya	Yenikoy/ Darende	Culban	1600
26	LS-4402		Yenikoy/ Darende	Culban	1600
27	LS-4403		Basdirek/ Darende	Culban	1445
28	LS-4404*		Commercial	Culban	-
29	LS-5001	Nevsehir	Kalecik/ Kozakli	Murdumuk	1120
30	LS-5002		Sadik/ Hacibektas	Murdumuk	1159
31	LS-5003		Sahinler/ Gulsehir	Murdumuk	880
32	LS-5004		Hacıhalilli/ Gulsehir	Murdumuk	1000
33	LS-5005		Cullar/ Acigol	Murdumuk	1086
34	LS-5006		Tatlarin/ Aciöl	Murdumuk	1113
35	LS-5501	Usak	Degirmenci/ Kavak	Feslek	600
36	LS-6401		Hacim/ Sivasli	Murdumuk/Fasil	938
37	LS-6402		Kasbelen/ Merkez	Murdumuk/Fasil	960
38	LS-6403		Kasbelen/ Merkez	Murdumuk/Fasil	960
39	LS-6404		Kasbelen/ Merkez	Murdumuk/Fasil	960
40	LS-6405		Inay/ Ulubey	Murdumuk/Fasil	743
41	LS-6406		Inay/ Ulubey	Murdumuk/Fasil	743
42	LS-6407		Inay/ Ulubey	Murdumuk/Fasil	743
43	LS-6408		Kisla/ Ulubey	Murdumuk/Fasil	800
44	LS-6409		Kisla/ Ulubey	Murdumuk/Fasil	800
45	LS-6410		Kisla/ Ulubey	Murdumuk/Fasil	800
46	LS-6411		Karacaahmet/ Ulubey	Murdumuk/Fasil	780
47	LS-6412		Ilyasli/ Merkez	Murdumuk/Fasil	770
48	LS-6413		Ilyasli/ Merkez	Murdumuk/Fasil	770
49	LS-6414		Ilyasli/ Merkez	Murdumuk/Fasil	770
50	LS-6415		Ilyasli/ Merkez	Murdumuk/Fasil	770
51	LS-6416		Ilyasli/ Merkez	Murdumuk/Fasil	770
52	LS	Released variety 'Gurbuz-2001'			-
53	LC-4801	Mugla	Cumalı- /Datça	Kara murdumuk	?

*Commercial material; ** first two numbers are city traffic code and last two numbers are sample number; LS, *L. sativus*; LC, *L. clymenum*.

Table 2. Local use, morphological and chemical features of accessions of *Lathyrus* sp.

Accession*	Local Use	Seed coat color***	1000 seed weight (g)	Crude protein (%)	β-ODAP (mg g^{-1})
LS-0201	Feed	G+B	92.0	30.90	1.90
LS-0202	Feed	G+B	84.9	28.03	1.66
LS-1501	Feed+food	G	110.6	30.04	2.19
LS-1502	Feed+food	G+B	86.4	29.00	1.75
LS-1503	Feed+food	G+B	94.4	30.09	3.24
LS-1601	Feed+food	G+B+C	102.3	30.57	1.68
LS-1602	Feed+food	C+B	134.0	28.00	2.32
LS-1603	Feed+food	G+B	107.3	28.61	2.38
LS-1604	Feed	G+B	99.6	27.61	2.62
LS-1801	Feed	G+B	97.8	27.98	1.83
LS-1802	Feed	G+B	122.6	27.50	2.06
LS-1803	Feed	B	104.7	28.50	3.86
LS-2001	Feed	G+B	76.3	29.34	2.30
LS-2002	Feed	G+B	80.6	30.10	2.07
LS-2003	Feed	G+B	121.5	26.96	2.02
LS-2004	Feed	G+B	115.0	26.67	3.33
LS-2005	Feed	G+B	77.2	27.00	3.84
LS-2006	Feed	B	73.3	26.23	1.74
LS-2007	Feed	G+B	90.5	27.86	2.26
LS-2008	Feed	G+B	101.5	27.18	1.71
LS-2301	Feed	B	106.3	29.27	1.74
LS-2302	Feed	B	72.2	27.34	3.15
LS-2303	Feed	G+B	89.94	27.80	2.25
LS-4301	Feed	G+B+C	118.6	30.41	1.81
LS-4401	Feed	G+B	80.8	30.33	1.41
LS-4402	Feed	G+B	101.8	28.62	1.99
LS-4403	Feed	B	103.3	28.16	2.02
LS-4404	Feed	G	129.0	27.02	2.67
LS-5001	Feed	G+B	106.5	28.55	2.18
LS-5002	Feed	G+B	102.2	28.69	2.20
LS-5003	Feed	G+B	112.2	27.99	2.00
LS-5004	Feed	G+B	103.4	27.98	2.07
LS-5005	Feed	G+B	140.8	26.23	2.93
LS-5006	Feed	B	113.6	28.80	2.06
LS-5501	Food	C	148.0	24.07	1.80
LS-6401	Feed	G+B	88.3	28.77	1.54
LS-6402	Feed	G+B	95.4	28.21	1.42
LS-6403	Feed	G+B	107.3	27.91	1.89
LS-6404	Feed	G+B	92.2	27.63	1.85
LS-6405	Feed	G+B	108.9	29.19	1.72
LS-6406	Feed	G+B	75.3	29.78	1.35
LS-6407	Feed	G+B	107.5	28.60	2.42
LS-6408	Feed	G+B	79.7	28.32	2.59
LS-6409	Feed	G+B	76.8	26.60	2.36
LS-6410	Feed	G+B	104.0	27.63	2.32
LS-6411	Feed	G	85.4	27.78	1.91
LS-6412	Feed	G+B	93.4	28.09	1.71
LS-6413	Feed	G+B	97.1	27.61	2.38
LS-6414	Feed	G+B	85.0	29.70	2.65
LS-6415	Feed	G+B	90.3	29.51	1.69
LS-6416	Feed	G+B	86.7	29.15	2.90
LS**	Feed	B	117.8	25.81	2.50
LC-4801	Food	B	118.8	26.33	2.68

* LS, *L. sativus*; LC, *L. Clymenum*; ** released variety; *** G, grey; B, brown; C, creamy.

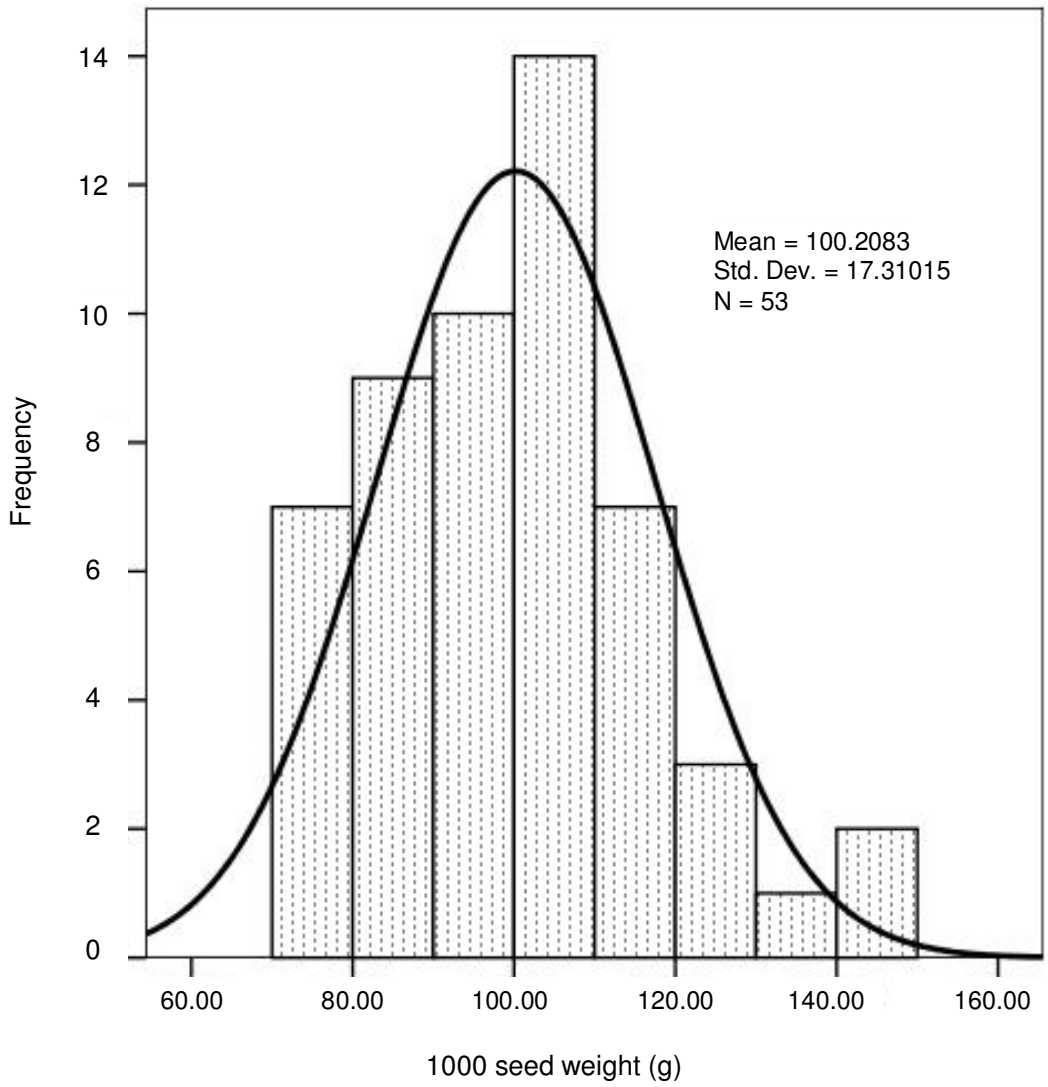

Figure 2. General distribution of *Lathyrus* landraces for 1000 seed weight.

β-ODAP levels of the samples were between 1.35 and 3.86 mg g^{-1} (Table 2) with a mean of 2.21 mg g^{-1} (Figure 4.) The highest toxic level was determined in the landrace collected from Cankiri numbered 1803, while the lowest level was obtained in the landrace collected from Usak numbered 6406 (Table 2). The β-ODAP concentration in the released variety named 'Gurbuz- 2001' was higher than the mean of all the samples (2.50 mg g^{-1}). Low toxic levels generally, were in landraces belonging to *L. sativus* and β-ODAP content was determined as 2.68 mg g^{-1} in *L clymenum* seed (Table 2).

β-ODAP concentration of *L. sativus* seeds varied between 0.2 to 7.2 mg g^{-1} in dry matter (Deshpande and Campbell, 1992). For safe consumption by humans, β-ODAP content of seeds has to be lower than 2.2 mg g^{-1} (Abd El Moneim et al., 1999). In the present work, β-ODAP was lower than 2.00 mg g^{-1} in 21 *L. sativus* landraces (Figure 3), indicating the importance of landraces in screening for low toxic content.

There has been no lathyrism case documented in human or animals up till now in Turkey. Also, farmers did not know anything about lathyrism or any toxic effect of *Lathyrus* seeds on human or animals. In fact, lathyrism risk is very low in Turkey. This is probablly due to low and rare consumption, soaking procedure and low β-ODAP content, because, the grain of *Lathyrus sativus* is rarely used in human diet for stock feeding in the country. Neurotoxic β-ODAP is a water-soluble amino acid that can be leached from seed by soaking in water (Akalu et al., 1998). On the other hand, Lahyrism is induced by heavy and prolonged (more than three months) consumption of the *L. sativus* seeds (Mehta et al., 1994).

Genotype is the most important factor determining ODAP concentration; environment has less influence (Hanbury et al., 1999). However, environmental factors such as drought, zinc deficiency, iron oversupply and the

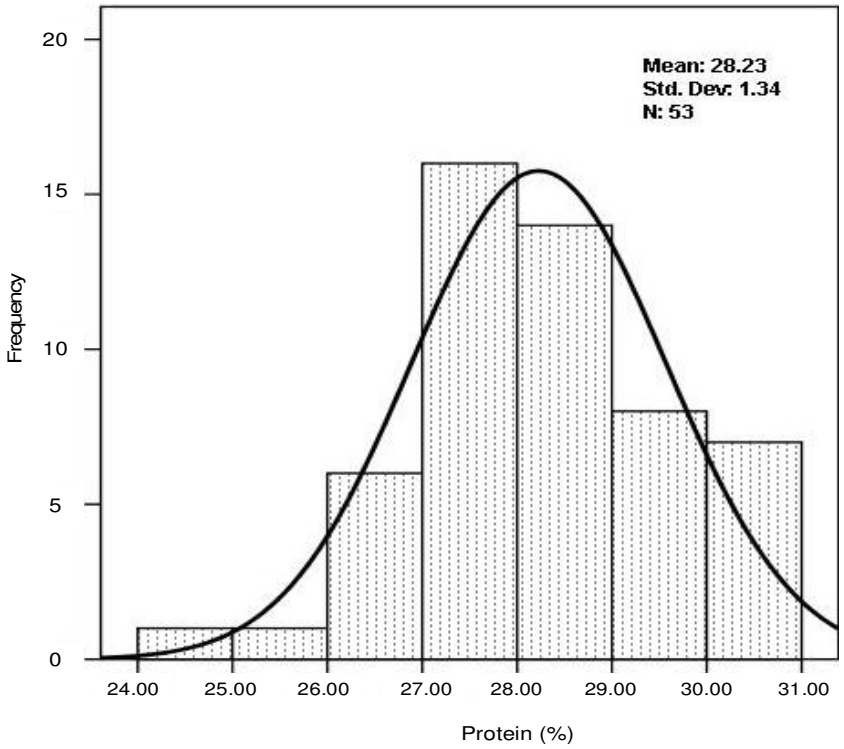

Figure 3. General distribution of *Lathyrus* landraces for seed protein contents.

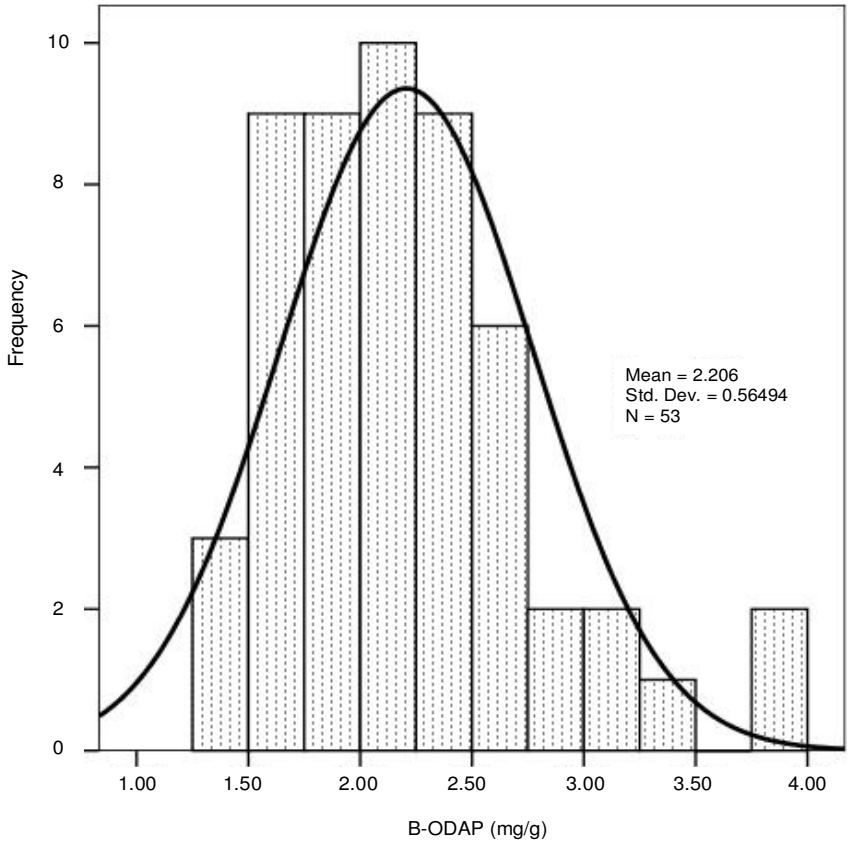

Figure 4. General distribution of *Lathyrus* landraces for seed β-ODAP contents.

presence of heavy metals in soil can considerably increase the level of β-ODAP in seeds (Lambein et al., 2007). So, the development of varieties for a specific region is one of the effective solutions to avoid lathyrism. At this point, local landraces will be usefull. Analysis of a large number of germplasm of *L. sativus* showed that, samples originated from Africa, Syria, Turkey and Cyprus had significantly lower content in the dry seed than in the samples originated from Bangladesh, Ethiopia, India, Nepal and Pakistan (Abd El Moneim et al., 2001). Therefore, the variation in this study on β-ODAP level was attributed both to genetic factors and environmental conditions. For this reason, there are needs for new studies, to determine the β-ODAP levels of these landraces in different ecological conditions.

L. sativus, which is a drought tolerant legume, is a promising crop for the diversification of cropping systems and sustainability of agriculture in marginal and drought areas. Furthermore, under the risks of climate change which is supposed to cause serious drought, the plant will become a more important crop in the world. So, future studies should concentrate on developing new varieties with high yield, low β-ODAP and high protein level. The results of this showed that, *L. sativus* landraces cultivated in Turkey, are promising genetic materials especially with their low β-ODAP contents.

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