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VARIATION AND CORRELATION OF MORPHO-AGRONOMIC TRAITS AND BIOCHEMICAL CONTENTS (PROTEIN AND B-ODAP) IN TURKISH GRASS PEA (*Lathyrus sativus* L.) LANDRACES

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ABSTRACT

Fifty-one landraces and one released variety 'Gurbuz-2001' of grass pea (*Lathyrus sativus* L.) collected from Turkey and grown in Samsun-Turkey condition were characterized by morpho-agronomic traits, protein and neurotoxic β-ODAP (β-*N*-oxalyl-L-*α*,β-diaminopropionic acid) contents in seed. High variation was observed among landraces regarding all the studied traits. β-ODAP content was determined by using capillary zone electrophoresis (CZE) and extreme levels of β-ODAP were 1.40 and 3.05 mg g^{-1} in among the landraces (average 1.96 mg g^{-1}). Also the correlations between traits were investigated and, the strongest positive correlations were between main stem number and leaflet length and, between seed yield per plant and pod number per plant. Seed yield significantly ($p< 0.01$) and positively correlated with most of the investigated traits. Correlations between β-ODAP content and other traits were negative or no significant. In general, many of the landraces were superior than released variety 'Gurbuz-2001' with their seed yield, protein content and β-ODAP content.

Key words: β-ODAP, characterization, landrace, *Lathyrus sativus*

INTRODUCTION

Recently, in response to serious concerns about food security and agricultural sustainability especially due to global climatic changes, some neglected or under-utilized plants that are nutritionally rich and adapted to difficult environments and low input agriculture such as grass pea (*Lathyrus sativus* L.) are increase in importance in the world (Kumar et al., 2011). *Lathyrus* genus (*Fabaceae*) includes about 150 species (Kupicha, 1983), however, only a few species are economically important as food, feed and forage (Campbell 1997) Grass pea is the most important and widely cultivated species of *Lathyrus* genus as pulse (Siddique et al., 1996). Grass pea is an annual legume crop with high protein content and significant resistance to extreme environmental conditions including marginal areas with poor soil fertility and water logging and in areas receiving only 380–650 mm annual rainfall (Girma and Korbu 2012). It is also resistance to many diseases and pests compared to other legumes (Tiwari and Campbell 1996). Thus, it has been renewed interest in drought- prone and marginal agro environments in the world over the recent decades. (Vaz patto et al., 2006). Despite its superior properties, using of grass pea has been restricted by the presence of anti nutritional factors particularly of the neurotoxic β-ODAP (β-*N*-oxalyl-L-*α*,β-diaminopropionic acid) in the seed. β-ODAP is considered to be reason for 'neurolathyrism' which is causing paralysis of the limbs (Rao et al., 1964). Lathyrism occurs when seeds are taken in large quantity in diet for an extended period of 3–4 months (Getahun et al., 1999). For this reason, grass pea breeding has mainly focused on improving cultivars with high yield and low β-ODAP content in all over the world (Vaz Patto et al., 2006). The β-ODAP content in grass pea seeds is highly influenced by genotype, environment and their interactions (Hanbury et al., 1999) and seeds having less than 0.2% ODAP are safe for consumption (Abd El Moneim et al., 1999). Many previous studies indicated a high variation in grass pea genotypes for β-ODAP content (Campbell et al. 1994; Tadesse and Bekele 2003; Hanburry et al. 1999; Karadag et al. 2010) and vegetative characters (Jockson and Yunus 1984; Campbell 1997).

Although it was widely cultivated in the past as forage, feed and food, grass pea is now rarely grown in Turkey. There is only one commercial varieties of grass pea in the country; therefore, almost all of the sowing seeds are

landraces types. Interestingly, no lathyrism disease was documented in Turkey (Basaran et al., 2011a) contrary to Many Asian, African and European countries. In addition Basaran et al. (2011b) who investigated a number of Turkish grass pea landraces grown their specific locations for 1000 seed weight, protein and β-ODAP content, reported that many of the landraces were promising for breeding. Turkish grass pea landraces have not been particularly characterized for morphological and agronomic traits up to now. Therefore, the aim of this study was to characterize Turkish grass pea landraces for morphological, agronomical and biochemical (β-ODAP and protein) traits and also to investigate the correlations between these traits.

MATERIALS AND METHODS

Plant material

Fifty-one Turkish grass pea (*Lathyrus sativus* L.) landraces and one released variety named 'Gurbuz-2001' were investigated. Landraces of grass pea were collected from 11 different provinces of Turkey (Adiyaman, Burdur, Bursa, Cankiri, Denizli, Elazig, Kutahya, Malatya, Nevsehir, Samsun, Usak) in 2007.

Field trials

Field experiments were conducted in Experiment Field of Agricultural Faculty of Ondokuz Mayis University located at Samsun (264972E - 4581185N, UTM), Turkey during 2007-2008 and 2008-2009 growing seasons (November-June) Slope is 2 % and altitude is 158 m.. Soil contents clay with approximately 2.93% organic matter, phosphorus content 22.89 ppm, potassium content 84.44 ppm, and pH 6.86. Long term (1974-2009) annual rainfall and mean temperature of experimental area 680.9 mm and 14.3 0C respectively.

Sowing was done by hand at 15 cm seed to seed and 30 cm row to row spacing in mid November in booth years. One plot (3 m length with 3 rows) was formed for each landrace. The following traits; days to flowering, plant height (cm), number of main stems per plant, leaflet length (cm), petiole length (cm), number of pods per plant, pod length (cm), number of seeds per pod, seed yield per plant (g), harvest index (%), 1000 seed weight (g), protein content (%) and β-ODAP content (%) were evaluated. Morphological measurements were performed on five plants randomly selected from each plot according to IPGRI (2000). The flower color was determined on the flag leaf of freshly opened flowers. Pod length and number of seeds per pod were evaluated on mature pods (5 pod per plant). Total nitrogen was determined by the Kjeldahl method and crude protein content was estimated using a conversion factor of 6.25 with three replications.

Determination of β -ODAP

β-ODAP content of landraces (three observation per landraces) was determined using capillary zone electrophoresis (CZE) (Zhao et al., 1999) with some modifications. CZE was carried out using Agilent HP3D 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 grass pea 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.

Statistics

This study was repeated two years and results were combined. The morphological and chemical traits among grass pea were presented as a mean, minimum, maximum, and standard deviation. Correlation analysis was also carried out to determine the associations between the investigated traits. The similarities among the grass pea landraces based on morphological and biochemical traits (protein and β-ODAP content in seed) evaluated by means of Principle Component Analysis (PCA). Statistical analysis were performed by using SPSS 13.0 Package Program

RESULTS

Results showed that there are high variation relations to investigated traits among the grass pea (*L. sativus*) landraces, even among the landraces those are originated same region (Table 1, 2). Among the landraces three flower color (blue, pink, white) were observed, as alone or mixture. The landraces were characterized by alone blue (39 landraces) or mixture of blue-white (4 landraces), blue-pink (7 landraces) and blue-white-pink (1 landrace) flower color. Only one landrace (S) was characterized by alone white flower (Table 1). Seed coat color was brown and cream and, the observations on plants indicated that there is a close relationship between seed coat and flower color. The plants having blue and pink flowers produced brown seed while white flowering ones were creamy seed (not given in tables). Days to flowering (days from sowing to beginning of the flowering) showed 15 days differences among grass pea landraces and, released variety (GR) was the earliest genotype with 160 days (Table 1, 3). However the variation in days to flowering was very low compared the other investigated traits in Turkish grass pea landraces. The highest variability was observed in seed yield per plant (CV= 30.80%) (Table 3). The extreme values for seed yield were 4.58 g (landrce M3) and 15.59 g (landrace N1) (Table 2, 3)

As a legume crop grass pea landraces exhibited a high protein content with a low variation (CV=2.58%). The highest protein content (25.04%) was recorded in landrace M3 which was also the lowest yielding landrace. In this study, no β-ODAP free landrace was detected. However

approximately all of them except two landraces had lower β-ODAP content compare to registered variety 'Gurbuz 2001', and also a number of landraces (42 landraces) were in the safe range (< 2.0 mg g-1) for human consumption regarding this toxic compound (Table 2).

Table 1. Origin and mean values of some morphological traits of Turkish grass pea landraces

Landraces and locations		Flower color	Days to flowering (day)	PH (cm)	NMS	PTL (cm)	LL (cm)
A1	Adiyaman	Blue	164	31.90	6.40	1.42	3.91
A2		Blue	164	36.53	12.30	1.67	6.99
B1	Burdur	Blue	164	32.36	9.30	1.54	5.42
B2		Blue	171	37.05	8.10	2.00	5.05
B3		Blue-white	169	37.59	8.70	1.80	5.25
BR1	Bursa	Blue- white	171	35.51	11.40	1.82	6.61
BR2		Blue- white	170	45.46	9.60	2.20	5.90
BR3		Blue	169	38.45	8.00	1.87	4.93
BR4		Blue	170	48.45	13.60	1.98	5.58
C1	Cankiri	Blue	169	39.83	9.40	1.62	5.51
C2		Blue	165	38.05	8.30	1.66	4.98
C3		Blue-white-pink	167	36.84	12.00	1.63	6.81
D1	Denizli	Blue-pink	167	33.31	7.70	1.68	4.69
D2		Blue	165	39.66	8.30	1.79	5.05
D3		Blue-pink	166	30.22	8.90	1.49	5.19
D4		Blue	164	39.78	10.70	1.80	6.25
D5		Blue	167	31.76	6.80	1.39	4.09
D6		Blue	166	32.52	7.40	1.42	4.41
D7		Blue	165	34.43	8.20	1.27	4.73
D8		Blue	165	34.54	9.40	1.49	5.44
E1	Elazig	Blue	162	41.81	10.90	1.92	6.41
E2		Blue	166	36.03	9.00	1.40	5.20
E3		Blue	167	32.06	7.10	1.35	4.23
K	Kutahya	Blue-white	167	41.88	8.90	1.60	5.25
M1	Malatya	Blue	163	38.30	8.10	1.81	4.96
M2		Blue	162	45.67	10.00	1.71	5.86
M3		Blue	162	38.75	6.60	1.69	4.15
M4		Blue	162	44.29	9.00	1.75	5.37
N1	Nevsehir	Blue	164	50.43	8.60	1.89	5.24
N2		Blue	164	56.00	8.60	1.92	5.26
N3		Blue	164	40.80	10.40	1.79	6.09
N4		Blue	165	41.73	9.80	1.81	5.81
N5		Blue	164	33.25	9.60	1.72	5.66
N6		Blue	165	41.18	7.40	1.85	4.62
S	Samsun	White	175	40.16	8.80	2.36	5.58
U1	Usak	Blue	171	30.14	6.30	1.40	3.85
U2		Blue	167	33.69	6.40	1.49	3.94
U3		Blue	162	39.24	6.10	1.63	3.87
U4		Blue	164	43.74	8.90	1.90	5.40
U5		Blue-pink	168	35.62	7.90	1.74	4.82
U6		Blue-pink	166	34.21	7.70	1.63	4.66
U7		Blue	169	33.96	8.30	1.76	5.03
U8		Blue	167	32.44	8.40	1.52	4.96
U9		Blue	169	34.60	6.20	1.52	3.86
U10		Blue	167	34.46	8.80	1.67	5.24
U11		Blue-pink	167	34.81	6.20	1.58	3.89
U12		Blue	170	31.13	11.10	1.72	6.41
U13		Blue-pink	166	34.62	10.90	1.60	6.25
U14		Blue	167	36.58	9.10	1.66	5.38
U15		Blue-pink	169	34.46	9.70	1.74	5.72
U16		Blue	169	32.55	7.60	1.52	4.56
GR*	-	Blue	160	33.56	7.90	1.66	4.78

PH; plant height, NMS; Number of the main stem, PTL; petiol length, LL; leaflet length

Table 2. Mean values for some traits of Turkish grass pea landraces.

Landracesa Accession	NPP	PL (cm)	NSP	SY (g/plant)	HI (%)	TSW (g)	SPC (%)	β-ODAP (mg. g^{-1})
A1	21.10	3.04	3.50	6.23	48.09	115.00	22.99	2.60
A2	45.00	2.92	3.40	13.58	51.60	131.00	24.20	2.30
B1	22.40	3.13	3.40	7.50	47.59	97.58	23.10	2.20
B2	35.40	3.35	3.60	15.00	51.38	103.02	23.97	1.95
B3	30.50	3.44	3.60	10.70	49.54	109.05	22.75	1.85
BR1	34.80	3.34	3.40	15.45	50.59	139.43	23.87	1.55
BR2	33.40	3.32	3.45	12.76	50.19	135.03	23.55	1.60
BR3	29.80	3.14	3.40	8.48	39.76	135.05	22.68	1.80
BR4	34.00	2.90	3.85	10.59	41.26	121.13	23.79	2.10
C1	26.70	3.10	3.95	9.17	45.34	100.23	23.50	1.80
C2	31.00	3.14	3.30	10.16	50.11	102.08	23.84	1.70
C3	31.60	3.20	3.65	12.10	50.73	107.93	23.62	1.40
D1	29.90	3.24	3.65	11.23	49.49	104.83	24.01	2.10
D2	22.50	3.09	3.15	7.64	48.42	106.63	24.67	1.60
D3	22.90	3.23	3.75	7.88	48.84	120.95	23.42	1.85
D4	38.50	3.24	3.80	13.66	53.79	127.10	22.36	1.60
D5	17.00	3.13	3.55	5.71	48.50	116.27	22.44	1.75
D6	20.60	3.05	3.05	7.45	48.79	111.80	23.64	2.15
D7	23.60	3.21	3.85	9.49	48.92	120.30	23.71	1.90
D8	26.00	3.07	3.40	10.33	53.95	125.88	23.49	1.70
E1	34.20	3.34	3.65	14.02	48.74	131.35	24.24	1.50
E2	19.70	2.91	3.85	5.67	43.04	94.20	22.99	2.40
E3	17.60	2.73	3.25	4.61	44.14	79.93	24.32	3.05
K	29.80	3.57	3.20	11.52	50.96	152.13	24.29	1.75
M1	29.80	3.09	3.50	8.19	46.28	108.23	22.69	2.15
M2	27.30	3.15	3.51	10.13	46.28	107.28	23.09	2.00
M3	15.80	2.97	3.60	4.58	46.68	101.43	25.04	2.00
M4	20.10	3.26	4.10	7.69	49.09	116.90	24.00	2.85
N1	41.40	3.33	3.80	15.59	50.81	110.25	24.33	1.50
N2	35.80	3.26	3.80	13.13	46.92	109.35	23.82	1.50
N3	31.00	3.03	3.50	9.12	44.71	111.85	23.21	1.70
N4	25.20	3.05	3.50	9.21	47.78	106.95	22.42	1.65
N5	19.70	2.94	2.90	6.25	41.11	128.10	23.97	1.95
N6	21.20	3.03	3.95	8.62	50.46	119.05	23.80	2.30
S	36.80	3.51	3.10	13.20	43.43	116.60	23.96	2.30
U1	19.20	3.02	3.25	6.07	46.02	115.10	23.77	2.15
U2	15.40	3.23	3.75	4.63	48.89	107.63	23.55	2.05
U3	21.50	3.17	3.45	8.22	52.55	107.90	23.23	1.75
U4	26.00	3.03	3.95	8.64	51.99	105.60	23.83	1.85
U5	25.80	2.98	3.60	8.42	48.54	107.18	23.72	2.35
U6	25.90	3.22	3.80	9.79	52.28	103.45	23.66	2.10
U7	29.10	3.02	3.20	7.73	43.59	109.05	24.27	2.10
U8	27.40	3.15	3.65	8.53	50.80	99.90	23.41	2.15
U9	14.40	3.14	3.55	4.97	49.85	96.45	21.96	1.90
U10	32.00	2.91	3.25	10.98	50.19	110.33	23.48	1.95
U11	21.50	3.31	3.90	7.51	50.15	110.18	23.23	1.70
U12	26.40	2.93	3.35	8.76	46.50	103.18	23.47	1.80
U13	29.90	3.12	3.85	10.37	50.03	101.98	24.08	1.85
U14	30.00	2.91	3.35	10.03	47.45	105.18	24.19	1.85
U15	32.50	3.20	3.60	10.56	49.05	105.45	23.74	2.00
U16	20.50	3.16	3.35	6.45	46.23	102.13	23.61	1.85
GR*	21.50	3.21	3.50	6.73	46.37	113.01	23.29	2.65

NPP; number of the pod/plant, PL; pod length, NSP; number of the seed /pod, SY; seed yield/plant, HI; harvest index, TSW; thousand seed weight, SPC; seed protein content.

Table 3. Mean values, ranges and coefficient of variation among Turkish grass pea landraces for each trait analyzed

Traits	Mean	Min	Max	SD	CV
Plant height (cm)	37.43	30.14	56.00	5.35	14.30
Number of main stem	8.75	6.10	13.60	1.66	18.29
Petiole length (cm)	1.69	1.27	2.36	0.21	12.50
Leaf length (cm)	5.17	3.85	7.00	0.80	15.47
Number of the pod/plant	26.94	14.40	45.00	6.90	25.61
Pod length (cm)	3.14	2.73	3.57	0.17	5.41
Number of the seed/pod	3.54	2.90	4.10	0.26	7.34
Seed yield / plant (g)	9.32	4.58	15.59	2.87	30.80
Harvest index (%)	48.23	39.76	53.95	3.15	6.53
Thousand seed weight (g)	112.06	79.93	152.13	12.76	11.39
Seed protein content (%)	23.58	21.96	25.04	0.61	2.58
β-ODAP (g mg^{-1})	1.96	1.40	3.05	0.34	17.35
Days to flowering (day)	166	160	175	2.97	1.79

Correlation analysis indicated that there are significant associations between the investigated traits (Table 4), The strongest positive correlations were between main stem number and leaflet length and between seed yield per plant and pod number per plant. Seed yield significantly ($p< 0.01$) and positively correlated with most of the investigated traits. In general, correlations between β-ODAP content and other traits were negative or no significant.

Results of PCA based on means of evaluated morphological and chemical traits was given in Table 5. The first two components explained 48.67% of total

variation (34.53 and 14.13% respectively). In the first component the traits with the most important contribution were about to yield, β-ODAP and those are highly and positively correlated with yield. Second component (14.13% of variability) mainly loaded by pod length and harvest index. The obtained scatter plot using first two principal components is shown in Figure 1. Considering the distribution of the landraces on the scatter plots, it can be concluded that there are remarkable differences among Turkish grass pea landraces, even among the landraces collected same region regarding investigated morphological and quality traits. Also this distribution released that many of the landraces were superior than released variety (Gurbuz-2001) with their seed yield, protein content and low β-ODAP percentage.

Table 4. Correlation among 13 different characters of Turkish grass pea landraces

Traits	PH	NMS	PTL	LL	NPP	PL	NSP	SY	HI	TSW	SPC	β -ODAP
PH	1											
NMS	0.307*	1										
PTL	0.636**	0.369**	1									
LL	0.291*	0.931**	0.440**	1								
NPP	0.464**	0.643**	0.613**	0.695**	1							
PL	0.256	-0.044	0.349*	0.077	0.304*	1						
NSP	0.329*	0.072	-0.001	0.011	0.022	0.149	1					
SY	0.487**	0.581**	0.600**	0.660**	0.916**	0.496**	0.095	1				
HI	-0.011	-0.053	-0.095	0.052	0.180	0.350*	0.276*	0.369**	1			
TSW	0.102	0.097	0.248	0.176	0.202	0.598**	-0.204	0.421**	0.288*	1		
SPC	0.193	0.142	0.174	0.148	0.254	-0.022	-0.007	0.219	0.016	0.046	1	
β –ODAP	-0.273	-0.247	-0.266	-0.250	-0.341*	-0.258	0.006	-0.379**	-0.091	-0.304*	0.077	1
DFL	-0.273*	0.048	0.212	0.106	0.267	0.145	-0.300*	0.203	-0.079	0.026	0.019	-0.091

*, **; Correlation is significant at the 0.01 and 0.05 level respectively

PH; plant height, NMS; number of the main stem, PTL; petiole length, LL; leaflet length, NPP; number of the pod/plant, PL; pod length, NSP; number of the seed /pod, SY; seed yield/plant, HI; harvest index, TSW; thousand seed weight, SPC; seed protein content, DFL; days to flowering.

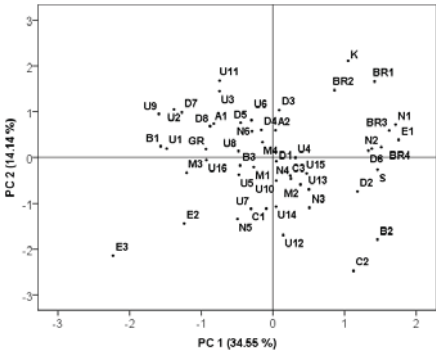

Figure 1. Results of the principle component analysis and dispersion of the grass pea landraces on the plot of the first-three components.

Table 5. Correlation of the analyzed traits with firs two component

Traits	Component	
	1	2
PH	0.604	-0.021
NMS	0.710	-0.516
PTL	0.736	-0.083
LL	0.774	-0.415
NPP	0.898	-0.150
PL	0.480	0.721
NSP	0.103	0.171
SY	0.936	0.113
HI	0.226	0.618
TSW	0.435	0.589
SPC	0.248	-0.193
β -ODAP	-0.476	-0.193
DFL	0.176	-0.074
% of Variation	34.55	14.14

DISCUSSION

Morphological traits

Grass pea shows great morphological variation, however, exhibits two main distinct group; the blue-flowered types from south-west Asia, Ethiopia and the Indian subcontinent and the white and white-blue flowered types from Mediterranean basin (Jackson and Yunus 1984; Smartt 1984). A flower color in this species can be blue, pink, red, white or their combinations and there is close relationship between flower color and seed color; white flowers usually produces white to creamy seeds, whereas colored flowers are associated with colored seeds (Campbell 1997). Similarly our results suggested that seed coat color was dependent on the flower color; white flowered plants had cream seed coat and, blue or pink flowered plants had brown seed coat. Turkish grass pea landraces predominantly exhibited blue or lesser pink flowers with a one exception as alone white. This picture indicating that Turkish grass pea landraces have the characteristics of both origins regarding flower and seed color, but mostly of south-west Asia, Ethiopia and the Indian subcontinent.

Previous studies indicated that grass pea had a great variation regarding flowering time depending on genotype and ecology. Number of the days to flowering ranged from 58 to 66 in 31 accessions of grass pea from different European countries (Grela et al., 2010), from 47 to 94 days in 1177 line (Campbell 1997), 104 to 129 days in 24 Indian genotypes (Kumari 2001) under spring showing. On the other hand, De la Rosa and Martin (2001) declared that day to flowering changed between 136 and 147 for

sixty Spanish landraces under autumn sowing. So, a longer period of flowering in the present study can be attributed to autumn sowing than genotype.

Plant height in grass pea greatly variable traits (Campbell 1997) and can be highly effected by ecological conditions (Grela et al., 2010), The earlier studies indicated that plant height of grass pea was between 31.4 and 67.4 cm in Poland (Rybinski et al., 2008), 46 and 153 cm in Turkey (Turk et al., 2007), 24.5 and 172 cm in Canada (Campbell 1997). In this respect, the plant height determined in the present study was in the limits of the species, but with lower range (30.14 -56.00 cm) (Table 3).

Number of the stem per plant which is important trait on yield (Campbell 1997) can reach up to 40 in grass pea (Kumar and Dubey 2001). In a study by Rybinski et al. (2008) the range of this trait for thirty two European accessions was from 4.00 to 6.46, and for sixty Spanish landraces maximum 4.50 (De La Rosa and Martin 2001). The higher results for branch number in present study (ranging between 6.10 and 13.00) (Table 3) compare to earlier studies is promising, However, it may be reason of autumn sowing which is promote vegetative growth.

Leaf consisting of two pairs of linear- lanceolate leaflets is one of the most variable part in grass pea (Jackson and Yunus 1984) and length of leaflet ranges between 5 and 7.5 cm (Campbell 1997), between 0.7 and 9.9 cm in 72 landraces (Polignano et al., 2005). In this respect, our results for leaflets length (ranging between 3.85 and 7.00 cm) (Table 3) were similar to earlier studies.

Kumar and Dubey (2001) reported that pod number per plant varied from 6.52 and 46.80 in sixty Spanish grass pea landraces. According to Pandey et al. (1997), this trait ranged between 7 and 257 in over hundred lines. Similarly, pod number per plant showed high variation in the present study; moreover it was one of the highest variable trait (CV= 25.61 %) (Table 3) in investigated grass pea landraces. Our findings for other reproductive traits, pod length and seed number per pod were in harmony with Polignono et al. (2005) and Rybinski et al. (2008) who reported that the range was between 2.7 - 5.2 cm and 3.2 – 4.7 cm for pod length and between 1 - 5 and 1.08 – 3.74 for seed number per pod respectively in grass pea genotypes.

Grass pea is mainly cultivated for its seed. So, to improve high yielding genotypes is very important in grass pea breeding. It was reported that the grass pea genotypes originated from Mediterranean and Europe had higher seed yield and also seed weight than those originated from Indian subcontinent (Cooks et al. 2000; Campbell 1997). Seed yield per plant was reported between 0.5 and 19.7 g for over hundred Indian line (Pandey et al., 1997) while it was between 7.20 and 21.19 g for 32 line (Ribinski et al., 2008) and between 7.0 and 214g for 76 line originated Europe (Polignano et al., 2005). High variation and also heritability was reported in grass pea for 1000 seed weight (Milczak et al. 2001; Turk et al. 2007). The differences between European-Mediterranean genotypes and Indian genotypes for seed weight were confirmed by different authors. Average 1000 seed weight of European genotypes of grass pea was found to be 344 g (120–660 g) for thirty-one accessions (Grela et al., 2010), and between 87 and 510 g with a mean of 271 g for 32 lines (Ribinski et al., 2008), however, it ranged from 34.5 to 225.9 g and average 86.8 g for 272 lines mostly from Turkey and North Africa (Robertson and Abd El Moneim 1995) and between 27-65 g for the lines from Nepal (Joshi 1997). In this respect, seed yield per plant (4.58 – 15.59 g) and 1000 seed weight (79.93 – 152.13 g) (Table 3) of Turkish grass pea landraces was lower than European lines

Biochemical traits (protein and β-ODAP)

The variation among Turkish grass pea landraces for protein content were not very high (CV= 2.58) (Table 3) compare to the range (14 to 34.3%) reported by Hanbury et al. (2000) and Rosa et al. (2000). The effects of genotype on protein content is important, however, it changes highly depend on soil and ecology. So, it can be possible to obtain higher protein content in investigated landraces under favorable growing condition than present values (average 23.58%) (Table 3) determined in no fertilizing condition. In all the world, due to the neuro toxic effects, the major target of grass pea breeding is reducing β-ODAP content in the seed. The percentage of β-ODAP in grass pea seeds shows high variation and is affected by both genetic and environmental factors (Lambien et al. 1993; Abd El Moneim and Cocks 1993), but mainly by genetic (Hanbury et al. 1999; Cocks et al. 2000). Previous studies indicated that no genotype of grass pea was β-ODAP free, although in several lines the β-ODAP content was low. ODAP contents in grass pea seed had high variation depend on genotype and environment and, it ranged from 0.02 to 7.2% (ICARDA, 1995; Deshpande and Campbell 1992). The safe level of this toxic compound for human consumption is <0.2% (Abd El Moneim et al. 1999). In this respect, our results are highly promising with the low β-ODAP concentration as low as 1.4 mg g^{-1} (Table 3). Most of the investigated grass pea landraces (42 of 52 landraces) were in the safe range (< 2.0 mg g^{-1}) On the other hand β-ODAP content of registered variety (2.65 mg g^{-1}) was above the safe level (Table 2). Correspondingly, ICARDA, (1995) reported that the grass pea saples originating from Turkey (IFLLS 516, IFLLS 563 were found low β-ODAP (<0.07%) compare to samples originating Bangladesh, Ethiopia, India, Nepal, and Pakistan. For these reasons, new collection and characterization studies in Turkey would be highly important to identify genotypes with low ODAP content. On the other hand, different flower and seed colors in the same landraces and also our visual observations is support to possibilities of intra-landraces variation. So it may be possible to identify genotypes with lower β-ODAP by intra-landraces selection programs.

Correlation and Principle Component Analysis (PCA)

The correlation analysis showed that there is no significant association between β-ODAP and morphological traits (Table 4). The correlation of β-

ODAP content with yield and yield correlated traits such as pod number/plant and thousand seed weight were significant and negative. This result may be promising to improve new varieties with high yielding and low β-ODAP content. However, Cocks et al. (2000) who reported a negative relationship between ODAP concentration and the total amount of ODAP in the plant (total seed weight x ODAP concentration), proposed that low toxin concentration in plant with high seed yielding were caused by toxin dilution. So, the negative and significant correlation between β-ODAP and yield should be taken into consideration with this aspect by the breeders. The association between β-ODAP and Protein content was not significant. Similar results were reported by Tadesse and Bekele (2003). According to same authors, lack of correlation between the β-ODAP and protein indicates that ODAP is synthesized and stored independent of protein content.

Seed yield per plant significantly ($p< 0.01$) and positively correlated with most of the investigated traits especially with pod number per plant, length of leaflet and petiole, number of main stem (Table 4). Likewise, Kumar and Dubey (2001) reported that seed yield strongly associated with pod number per plant. Especially, positive and significant correlation of seed yield per plant with leaflet length and petiole length is highly promising. In addition, petiole length and leaflet length were positively correlated with protein content while they were negatively associated with β-ODAP. This result suggesting that these two traits (leaflet length and petiole length) could be used as selection marker especially for high seed yield and also maybe for high protein and low β-ODAP in early stage.

Seed yield was also positively associated with pod length and 1000 seeds weights and harvest index (Table 4). Likewise, a positive correlation of seed yield with 1000 seed yield was reported by Rybinski et al. (2008). Furthermore, Turk et al. (2007) found that 1000 seed weight affected at very low level by environment and they reported that it could be uses as selection criteria but harvest index not effective to high environment effects on it.

PCA analysis based on means of investigated traits showed that the association among the investigated was not very high, in fact the first two components explained only % 48.67 of the total variation (Table 5). This moderate percentage can be attributed to no specific breeding in Turkish grass pea landraces and wide ecological differences of collection area. De la Rosa and Martin (2001) who characterized sixty Spanish grass pea landraces for agro-morphological traits reported that first three components explained 54.96% of total variation. Same authors also declared that this low percentage, probably, due to the scarce breeding work made in Spanish populations. On the other hand, considering the distribution of the landraces on the scatter plots (Figure 1), it can be concluded that there is remarkable differences among Turkish grass pea landraces, even among the landraces collected same region regarding investigated traits. Also this distribution released that many of the landraces were superior than released variety 'Gurbuz-2001' with their seed yield, protein content and low β-ODAP percentage.

CONCLUSION

Consequently, in the present study a high amount of the morphological variation observed both inter and intra-landraces indicating the existent of a high genetic diversity in Turkey regarding grass pea germplasm. On the other hand, different flower and seed colors in the same landraces and also our visual observations is support to possibilities of intra –landraces variation. Also low neurotoxin (β-ODAP) content in many of the investigated landraces clearly indicated that they may provide a precious genetic material to improve low toxin varieties in breeding. However, these germplasm has been threatened by the serious genetic erosion resulted from decreasing in grass pea culture in Turkey. So, collection and preservation of them is very important in terms of the future of grass pea culture and breeding in Turkey and world as well.

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