Range Mgmt. & Agroforestry 40 (1) : 124-130, 2019
ISSN 0971-2070

Variation and association in *Lathyrus* species based on seed biochemical constituents

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Received: 10th April, 2018 Accepted: 20th April, 2019

Abstract
The seeds of twelve *Lathyrus* species were analyzed for crude protein, ODAP, ash, mineral contents and thousand seed weight. CP content varied from 20.78% in *L. cicera* to 30.92% in *L. ochrus*. ODAP content was the most variable trait (CV= 97.81%), being highest (10.31 mg g^{-1}) in *L. clymenum* (cultivar) and lowest (0.65 mg g^{-1}) in *L. laxiflorus* (wild). This was followed by thousand seed weight (TSW) being highest in *L. sativus* (114.54 g) and lowest in *L. nissolia* (3.45 g). These species also exhibited a reasonable variation regarding mineral contents, especially for Ca (CV= 40.29%) and Mn (CV= 40.96%). Correlation analysis, based on interspecies means indicated that ODAP was highly correlated with TSW (r = 0.762), Zinc (r= 0.732) and B (r=- 0.507), while there was no significant correlation of ODAP with CP and ash contents.

Keywords: Biochemical variation, *Lathyrus*, Mineral content, ODAP, Protein

Abbreviations: B: Boron; **Ca:** Calcium; **CP:** Crude protein; **Cu:** Copper; **Fe:** Iron; **K:** Potassium; **L.:** *Lathyrus*; **Mg:** Magnesium; **Mn:** Manganese; **ODAP:** â-N-oxalyl-l-á,â-diaminopropionic acid; **P:** Phosphorus; **TSW:** Thousand seed weight; **Zn:** Zinc

Inroduction

The *Lathyrus* is a large genus of Fabaceae with approximately 160 annual or perennial species distributed worldwide (Campbell, 1997). The main center of genus diversity is the Eastern Mediterranean region with lesser diversity in North and South America (Kupicha, 1983). Turkey has a high diversity for *Lathyrus* genus with 65 species, 23 of them endemic (Davis, 1970). A number of species have a long histoty as forage or fodder (*Lathyrus inconspicuous, L. ochrus*and, *L. articulates, L. hirsutus*, *L. palustris*, *L. sylvestris*), as food (*L. cicera and L. sativus, L. clymenum)* and some are valued as ornamentals, especially *L. odoratus* (Jackson and Yunus, 1984; Campbell, 1997). However, from an agricultural point of view, the most important species of the genus are *L. sativus*, *L. cicera* and *L. odoratus* being cultivated in more than 1.5 Mha of land worldwide (VazPatto and Rubiales, 2014).

Lathyrus species as fodder or seed crops exhibit great importance for sustainable plant production in alkaline, poor and water lodging soils and also in drought conditions. Nitrogen fixation, erosion control, high protein content, pest and disease resistance are additional advantages of *Lathyrus* species (Tiwari and Campbell, 1996; Grela *et al*., 2010). However, in spite of their high nutritional potential as protein source for populations in drought-prone and marginal areas, cultivation of *Lathyrus* is reducing and under the risk of genetic erosion (VazPatto and Rubiales, 2014).

Lathyrus is also an important source of minerals which is used as a forage crop for livestock in Mediterranean type environments (Skiba *et al.,* 2007; Polignano *et al.,* 2009). Basaran *et al.*(2016) reported that there was no significant difference in the yield and quality of grass pea compared with other legumes. Besides number of advantages, the drawback to the use of *Lathyrus* is the presence of the neurotoxin, â-N-oxalyl-l-á,â-diaminopropionic acid (ODAP) especially in seeds which can cause irreversible paralysis when it consumed in excess (Rao *et al.,* 1964; Singh and Rao, 2013; Mandal *et al.,* 2015). This toxic amino acid is found in *Lathyrus* seeds up to 1.6% (Sammour *et al.,* 2007), while it's safe level is than 0.2% for human consumption (Dahiya, 1976). ODAP was detected mainly in *L. sativus*, *L. cicera*, *L. clymenum*, *L. ochrus*, *L. hirsutus*, *L. sylvestris* and also with lesser content in *L. aphaca*, *L. sphaericus* and *L. gorgoni* (Siddique *et al.,* 1996; Sammour *et al.,* 2007; Basaran *et al.,* 2013). Therefore, to develop zero or low level ODAP content varieties is one of the main targets of *Lathyrus* breeding for safe consumption (Hanbury *et al.,* 2000). Cultivars containing very low ODAP ranging from

0.007%- 0.02% were found, however, no ODAP-free genotype was reported either from cultivars or wild species (Kumar *et al.,* 2011).

ODAP content changes depending on genetic and environmental factors (Abdel-Zaher *et al.,* 2007). Moreover, low toxin and high yielding varieties have been developed by hybridization, selection and genetic engineering (Abdel Moneim *et al.,* 1999; Hanbury *et al.,* 1999). Therefore, there is need to have comprehensive knowledge about genetic, morphological and chemical variation of *Lathyrus* resources, helping breeders to select promising genotypes to be used in breeding programs. Additionaly, knowledge of variation is useful in genebank management to create core collections and eliminate duplicates with efficient sampling (Radwan *et al.,* 2013). For these purposses, the present study was aimed to examine variation of ODAP, protein, ash, and mineral contents in *Lathyrus* sp.which included both cultivars and wilds.

Material and Methods

Plant materials: Seeds of the twelve *Lathyrus* species (Table 1), originating from Turkey were examined in terms of crude protein (CP), ash, thousand seed weight (TSW), neurotoxic â-N-oxalyl-l-á,â-diaminopropionic acid (ODAP) and minerals in terms of calcium (Ca), potassium (K), magnesium (Mg); phosphorus (P), iron (Fe), copper (Cu), zinc (Zn), manganese (Mn) and boron (B) contents.

Chemical and statistical analysis: Quantitative estimation of ODAP was performed by using the modified method of Rao (1978). Diaminopropionic acid (DAP) was used as a standard. For extraction, 20 mg finely powdered seed sample was taken in test tube and 2 ml of distilled water was added to it. The tubes were kept in boiling water and cooled to room temperature and then centrifuged. 100 µl of supernatant plus 0.2 ml of 3 N KOH were placed in tube and kept in boiling water for 30 minutes. After hydrolysis, 0.7 ml of water and 2 ml of OPT regent was added to tube and allowed to remain for 15 minutes for development of yellow colour. Finally, the yellow color was measured in spectrophotometer (Perkinelmer Lambda 25) at 425 nm. Total nitrogen was determined following Kjeldahl method and crude protein was estimated using a conversion factor of 6.25. Minerals were estimated using ICP- MS (Termo, ICAP- RQ). Data was analysed with SPSS 13.0 package program for descriptive statistics and, PCA analysis were performed based on all the investigated traits.

Results and Discussion

Chemical composition and thousand seed weight (TSW): The *Lathyrus* species exhibited high variation for most of the studied traits, which all had a coefficient variation of higher than 15% (Table 2-3). CP content of the species were ranged between 20.78% (*L. ochrus*) and 30.98% (*L. cicera*) with a medium coefficient of variation (CV= 12.44%). The highest variation among the investigated species was observed in ODAP content (CV = 97.81%) that was varied from 0.65 mg g^{-1} (*L. laxiflorus*) to 10.31 mg g^{-1} (*L. clymenum)* and averaged 3.32 mg g^{-1}. TSW was also more variable being highest in *L. sativus* (114.54 g) and lowest in *L. nissolia* (3.45 g). Ash content varied from 2.83 to 4.54% in *Lathyrus* species with a medium CV (13.60%). Mn and Ca exhibited higher variation with CV of 40.90% and 40.20%, respectively (Table 3). Fe was the lowest varaiable mineral (CV = 15%). Cu and Zn also had a high CV (29.70% and 28.93% respectively) while K, P, Mg and B exhibited medium and more or less similar coefficiant of variation.

Table 1. Geographical data, growing type and life form of the 12 *Lathyrus* species.

Species	Growing type	Life form	Location (Turkey)
L. aphaca L.	Wild	Annual	Samsun/ Merkez
L. hirsutus L.	Wild	Annual	Samsun/ Merkez
L. clymenum L.	Cultivar*	Annual	Mugla/Datca
L. laxiflorus L.	Wild	Perennial	Samsun/ Merkez
L. nissolia L.	Wild	Annual	Samsun/ Merkez
L. tuberesus L.	Wild	Perennial	Samsun/Kavak
L. annuus L.	Wild	Annual	Samsun/ Merkez
L. ochrus L.	Wild	Annual	Samsun/ Merkez
L. sphaericus L.	Wild	Annual	Samsun/ Merkez
L. cicera L.	Wild	Annual	Corum/Alaca
L. inconspicuous L.	Wild	Annual	Yozgat/Merkez
L. sativus L.	Cultivar**	Annual	-

*Landrace; **Released variety (Gurbuz-2001)

Lathyrus genus with about 160 anuual or perennial species exhibited high variation even intra-species level. This variation was explained in many earlier studies on the basis of morphological, agronomic, biochemical traits and genetic (Jackson and Yunus, 1984; Sammour *et al.,* 2007; Acar and Basaran, 2007; Emre *et al.,* 2015; Wang *et al.,* 2015). Among these 12 *lathyrus* species, protein content of seed was varied between 20.78 (*L. ochrus* - wild) and 30.92% (*L. cicera* -wild) and thousand seed weight varied from 3.45g (*L. nissolia*- wild) to 114.54 g (*L sativus* - cultivar) (Table 2). Sommour *et al.* (2007a) also reported that seed protein content was varied between 22.6 and 49.4%, while TSW was varied between 14 g and 315 g in 66 accessions belonging to 18 species of *Lathyrus*. Both protein and seed weight varied considerably in *Lathyrus* genus, even within species; Sommour *et al.* (2017b) observed that seed protein content ranged between 29 and 40% in eighteen *Lathyrus sativus* accessions. Similarly the range of thousand seed weight was between 57 and 315g (Sommour *et al.,* 2017b); 91 and 492g (Rybinsky *et al.,* 2008) in *L. sativus* genotypes. Thus *L. sativus* is the most important cultivated species all over the world and probably also the most variable species of the genus. This was usually an expected situation due to geographical distribution and also selection by man for thousands of years (Jackson and Yunus, 1984).

Table 2. Mean values of four quantitative traits studied in the 12 *Lathyrus* species

Species	CP (%)	ODAP (mg g^{-1})	TSW (g)	Ash (%)
L. aphaca	26.24	2.29	11.35	3.30
L. hirsutus	23.41	2.53	32.05	3.20
L. clymenum	27.46	10.31	108.55	3.55
L. laxiflorus	25.00	0.65	12.50	3.55
L. nissolia	30.75	1.92	3.45	4.54
L. tuberesus	27.05	1.03	39.70	3.89
L. annuus	21.34	1.77	51.65	3.78
L. ochrus	20.78	9.72	99.15	4.03
L. sphaericus	27.57	1.59	16.80	4.36
L. cicera	30.92	3.64	61.55	3.57
L. inconspicuous	29.22	1.15	14.40	2.82
L. sativus	26.94	3.25	114.54	3.25
Min	20.78	0.65	3.45	2.82
Max	30.92	10.31	114.54	4.54
Mean	26.39	3.32	47.14	3.65
CV (%)	12.44	97.81	85.60	13.60

CP: Crude protein; TSW: Thousand seed weight

No species was found ODAP free, although very low ODAP content of 0.65 mg g^{-1} was observed in the present study (Table 2). ODAP was the most variable trait (CV = 97.81%) being highest (10.31 mg g^{-1}) in *L. clymenum* (cultivar) and lowest (0.65 mg g^{-1}) in *L. laxiflorus* (wild). Similarly high coefficient of variation for ODAP (CV = 99%) was reported among 18 species of *Lathyrus* (Sommour *et al.,* 2007a). Besides the cultivar species (*L. clymenum* and *L. sativus*), *L. ochrus and L. cicera* had higher ODAP content and also higher seed weight. *L. ochrus* and *L. cicera* investigated are wild but have culture forms in Turkey and other parts of the world (Kendir, 1999). Especielly, *L. cicera* has been cultivated since ancient times. Moreover it is the most grown species of the genus after *L. sativus* in worldwide (Kislev, 1989). For safe human consumption ODAP should be lower than 2 mg g^{-1} (Abdel Moneim *et al.,* 1999). In this respect, it can be said that cultivars exhibited higher ODAP content than critical value (2 mg g^{-1}) while literally wild species had lower values. On the other hand, perennial species (*L. laxiflorus* and *L. tuberosus*) had lower ODAP content than that of annuals. Because ODAP is one of the most important factors that prevent the lathyrus cultivation (Lambein and Kuo 2009), thus devoloping low or ODAP free genotypes has become a goal of plant breeders for a long time (Kumar *et al.,* 2011). In this respect, seed size was perhaps the main factor in domestication of *Lathyrus* species in the past.

Ash content of the *Lathyrus* species studied was ranged from 2.82% (*L. inconspicuous*) to 4.54% (*L. nissolia*) (Table 2). This range was more or less similar to earlier studies. Aletor *et al.* (2011) who investigated twenty lines belonging three species reported that mean ash content was 2.96% in *L. cicera,* 3.89% in *L. sativus* and 3.45% in *L. ochrus.* Similarly it was observed that ash content varied from 3.4 to 3.9 g in *L.sativus* seeds depending on the ripeness of seeds (Lisiewska *et al.,* 2006).

The concentration of the minerals in the studied *Lathyrus* species were higly variable especially for Ca (CV= 40.29%) and Mn (CV= 40.96%) (Table 3). Among the maojor minerals, K and P were the most abundant in all speceis fallowed by Ca and Mg. Fe and Zn exhibited higher values when compared to other minor minerals. Similar ranking for *L. sativus*, *L. cicera* and *L. ochrus* was reported earlier by Aletor *et al.* (2011). However, they observed significantly lower P content compared to our values. No certain clustering regarding mineral content was observed among the species which included both cultivated and wild. However, with some exceptions, *L.*

Table 3. Mean values of minerals studied in the 12 *Lathyrus* species

Species	K (%)	P (%)	Mg (%)	Ca (%)	Fe (ppm)	Cu (ppm)	Zn (ppm)	Mn (ppm)	B (ppm)
L. aphaca	1.04	0.48	0.12	0.16	68.40	12.24	47.32	18.99	10.09
L. hirsutus	1.17	0.34	0.09	0.11	73.62	13.63	32.31	14.34	8.03
L. clymenum	0.79	0.47	0.11	0.14	67.36	8.45	56.04	11.73	9.60
L. laxýflorus	1.09	0.43	0.14	0.13	74.63	9.46	39.41	22.35	15.93
L. nissolia	0.89	0.43	0.11	0.19	53.00	17.24	36.54	39.96	15.02
L. tuberesus	0.89	0.39	0.10	0.10	65.97	12.55	28.87	13.65	13.82
L. annuus	1.07	0.37	0.10	0.20	70.20	9.49	35.17	20.24	9.74
L. ochrus	1.25	0.41	0.14	0.12	83.73	11.79	53.88	23.90	8.25
L. sphaericus	1.01	0.39	0.16	0.19	72.09	8.01	31.96	24.90	12.19
L. cicera	0.86	0.25	0.09	0.34	83.51	10.48	22.51	11.75	13.05
L. inconspicuous	0.88	0.23	0.09	0.12	77.97	7.86	23.43	12.55	11.64
L. sativus	1.51	0.50	0.15	0.13	97.56	18.46	41.41	20.46	15.48
Min	0.79	0.23	0.09	0.10	53.00	7.86	22.51	11.73	8.03
Max	1.51	0.50	0.16	0.34	97.56	18.46	56.04	39.96	15.93
Mean	1.04	0.39	0.12	0.16	74.00	11.64	37.40	19.57	11.90
CV (%)	19.57	21.45	22.14	40.20	15.00	29.70	28.93	40.96	23.42

Table 4. Correlations between quantitative traits in studied 12 *Lathyrus* species

	CP	ODAP	TSW	Ash	K	P	Mg	Ca	Fe	Cu	Zn	Mn	B
CP	1	-.245	-.255	.033	-.531*	-.205	-.177	.428	-.183	.097	-.437	.042	.564*
ODAP		1	.762**	.073	.025	.257	.117	-.082	.176	-.086	.732**	-.148	-.507*
TSW			1	-.119	.390	.281	.180	-.059	.588*	.164	.504*	-.304	-.202
Ash				1	-.169	.264	.376	.229	-.487	.124	.122	.717**	.175
K					1	.390	.543*	-.309	.674**	.526*	.270	.163	.027
P						1	.633*	-.368	-.097	.436	.771**	.401	.168
Mg							1	-.156	.294	.138	.442	.451	.282
Ca								1	.032	-.110	-.369	.054	.150
Fe									1	.099	-.010	-.382	.044
Cu										1	.107	.455	.341
Zn											1	.181	-.326
Mn												1	.338
B													1

*(P<0.05); **(P<0.01)

sativus exhibited higher values compared to others. Legumes generally have high mineral content (Sandberg, 2002). Mineral contents of the *Lathyrus* speceis studied were well comparable with the values reported by Souci *et al.* (2000) for common pulses such as common bean, lentil, chickpea, pea and soybean, with relatively lower Mg content. Additionally our results were in agreement with Urga *et al.* (2005), who studied mineral contents in 450 Ethiopian *Lathyrus sativus* samples.

Correlations analyses: The correlations were determined between the studied traits (Table 4). The CP content was highly correlated with K (r = -0.531), Ca (r = 0.428), Zn (r = -0.437) and B (r = 0.564) contents. Signi-ficant correlation of ODAP with TSW (r = 0.762), Zn (r = 0.732) and B (r = -0.507) were also recorded. TSW was higly correlated with Fe (r = 0. 588) and Zn (r = 0.504). Although ash is an indicator of total mineral content, it was correlated only with Fe and Mn. Moreover, the correlation between ash and Fe content was negative. On the ather hand significant and possitive correlations were observed between minerals. Especially correlation of K with Fe, and between P and Zn was vey high.

The correlation analyses preformed by using intra-species means indicated that there were no significant correlations among ODAP, CP and ash contents (Table 4). CP was positively accossiated with Ca and B, but it was negatively with K and Zn. ODAP was highly and possitvely correlated with TSW and Zn, while negatively with B. Ash content was correlated with only Zn and Fe, which indicated that *Lathyrus* species were rich in Zn and Fe, and also had high variation for mineral contents. Basaran *et al.* (2013) reported that the association of ODAP with CP was not significant in *Lathyrus sativus*. Similar result was reported by Tadesse and Bekele (2003), since ODAP and protein are independently synthesized and stored. Indeed, ODAP synthesis is higly varible with the environment effect. In *L. sativus*, high ODAP content was assosiated with Zn deficiency and Fe oversupply (Campbel, 1997). With the Zn fertilization, ODAP content was reduced in *L. sativus* seeds by 10-20% when compared to control group (Abdel-Moneim *et al.,* 2010). In contrast, based on eleven species means in the present study, ODAP showed high and positive (r = 0.732) assosiation with Zn, high and negative (r = -0.507) association with B, low and positive (r = 0.176) association with Fe. These conflicting results indicated that interactions between ODAP and minerals are complex and vary depending on species. So any obervations regarding ODAP x mineral interaction in certain species cannot be generalised for whole genus.

Biplot analyses: The scatter plot using first two components and distribution of the *Lathyrus* species was obtained (Fig 1). The association of the traits with the first two components was was also recorded (Table 5). The first component were loaded by protein (-0.532), ODAP (0.598), TSW (0.672) and minerals especially by K (0.714) and Zn (0.835). In the second component, the distribution of the species were mainly by the contribution of Mn (0. 878), B (0.638) and ash (0.699).

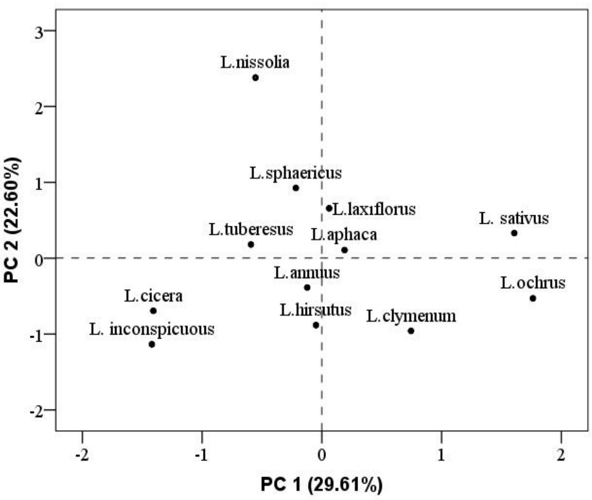

Fig 1. Biplot of principle component analysis of *Lathyrus* species based on thirteen quantitative traits

Table 5. Factor scores and contribution of the ûrst two principal components to the variation of 12 *Lathyrus* species

Traits	Component	
	PC1	PC2
CP	-0.582	0.305
ODAP	0.598	-0.386
TSW	0.672	-0.417
Ash	0.071	0.699
K	0.714	0.034
P	0.742	0.460
Mg	0.632	0.463
Ca	-0.445	0.089
Fe	0.387	-0.439
Cu	0.346	0.478
Zn	0.835	0.001
Mn	0.176	0.878
B	-0.203	0.638
% of variation	29.61	22.60

The first two principal components of PCA explained 52.21% (PC1 = 29.61% and PC2 = 22.60%) of the total variation. This moderate percentage indicated the high variation among the studied *Lathyrus* species on the basis of the studied traits. Considering the CP, ODAP and TSW, that are important traits for *Lathyrus*, PC1 allowed the more clear discrimination among species. Cultivar species (*L. sativus* and *L. clymenum*) and *L. ochrus* that are high in ODAP content and TSW grouped and located in possitive side on the PC1. Althougth having high ODAP content and TSW, *L. cicera* separated from this group and located in negative side due to higher CP and Ca content. The PC2 separation was mainly loaded by ash (0.699) and *L nissolia* was clearly differentiated from others with higher ash content (Fig 1).

Conclussion

The present investigation indicated the high diversity in 12 Turkey origin *Lathyrus* species based on the studied biochemical traits and seed weight. TSW and ODAP content were found as highly vriable traits. All the species exhibited high protein and satisfying mineral content indicating their potential to be used as food or feed. Although very low ODAP content was observed in some wild species, but no species was found ODAP free. However, high diversity among species and very low ODAP content in some of species revealed their importance in breeding programs to develop high quality and low toxin genotypes.

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