

Full Length Research Paper

Comparison of α , β and total ODAP (β -N-oxalyl-L- α , β -diamino propionic acid) contents in winter- and spring-sown grasspea (*Lathyrus sativus* L.) genotypes

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Accepted 12 May, 2010

There is a strong relationship between the consumption of grasspea (*Lathyrus sativus* L.) and “lathyrism” disease caused by a neurotoxin, β -N-oxalyl-L- α , β -diaminopropionic acid called ODAP or BOAA. The objective of this study was to compare α , β and total ODAP found in grasspea genotypes sown in winter and spring seasons during 2007/08 and grown under rainy conditions in semi-arid regions of Turkey. Biochemical compounds of α , β and total ODAP were found to be higher in spring-sown grass peas than those of winter-sown ones. Grasspea 452, 508 and 519 genotypes had lower β -ODAP levels in winter- and spring-sown.

Key words: Grasspea, α -ODAP, β -ODAP: 3-(-N-oxalyl)-L-2,3-diamino propionic acid.

INTRODUCTION

Grasspea has considerable potential as a forage crop in semi-arid regions (Abd El Moneim and Cocks, 1993; Siddique et al., 1996; Toker and Yadav, 2010). It is an important crop particularly in the South Asia and Ethiopia, with less intense production in China, Mediterranean region and Europe. It is grown for green feed and grain for both animal and human consumptions. Grasspea has an excellent reputation for its tolerance to waterlogging during the seedling stage, drought and consistent production in lower input environments (Campbell et al., 1994). Recent studies have shown that grasspea has substantial potential as a forage crop in Mediterranean-type environments of southern Australia (Siddique et al., 1996; Hanbury et al., 1999).

Beta-oxalyl-diamino-propionic acid (β -ODAP) is a neurotoxic metabolite present in grasspea (Bell, 2003; Mikic et al., 2009). Addis and Narayan (1994) studied plant developmental stage and relation to ODAP levels in different tissues. They have identified ODAP in roots and seeds during the developmental stages of grasspea plants. Frequent consumptions of grasspea seeds cause irreversible spastic paralysis of the legs known as neurolathyrism (Moges et al., 2004). There is a strong relationship between the consumption of grasspea and “lathyrism” disease caused by a neurotoxin, β -N-oxalyl-L- α , β -diaminopropionic acid called ODAP or BOAA (Campbell et al., 1994). The levels were influenced mainly by genotype (Hanbury et al., 1999). If ODAP level in a cultivar is below 0.2%, they are thought to be safe for human consumption (Dahiya, 1976). There are even epidemic cases during drought and food shortage periods (Moges et al., 2004).

The neurotoxic amino acid (β -ODAP) identification and quantification are the important phases in grasspea and require identifying the presence of nontoxic isomers and other possible interferences. The first method developed was Ninhydrin method. A colorimetric method was also

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Abbreviations: ODAP, β -N-oxalyl-L- α , β -diaminopropionic; RP-HPLC, reversed-phase high-performance liquid chromatography method; FDNB, 1-fluoro-2,4-dinitrobenzene.

developed and replaced by ninhydrin method (Rao, 1978). Colorimetric method is widely used for a long time. In this method, colorimetric determination of L- α , β -diaminopropionic acid (DAP) is possible (Moges et al., 2004). Later, a high-performance liquid chromatographic method was used to determine the neurotoxic amino acid. However, this method could also not differentiate β -ODAP from α -ODAP. Therefore, a new method, reversed-phase high-performance liquid chromatography method (RP-HPLC), has been developed to identify the α -ODAP and β -ODAP contents (Wang et al., 2000).

Many studies have been conducted to reduce the neurotoxic amino acids in grasspea lines. Some grasspea lines have been identified with lower neurotoxic amino acid contents (Sammour et al., 2007). However, there has been no solution to eradicate this problem fully until now because grasspea grows under adverse agricultural conditions that might change the chemical compounds of the plants (Roy, 1981; Milczak et al., 2001). New grasspea lines should be identified with lower neurotoxic amino acids. The objective of this study was to determine the α -ODAP and β -ODAP compounds of grasspea lines (*Lathyrus sativus* L.) seeds grown under rainy conditions in semi-arid regions of Turkey.

MATERIALS AND METHODS

This study was conducted at the Department of Field Crops of the Agricultural Faculty, Gaziosmanpasa University (Tokat-Turkey), (40° 13' - 40° 22' N, 36° 1' - 36° 40' E, altitude 623 m) in 2007 and 2008 growing season. Soil samples of 0 - 20 cm depths from both locations were collected to determine the initial conditions of experimental fields. The soils were slightly alkaline, medium in calcium carbonate and in P content, high in K and poor in organic matter content. Average temperatures of 9.0 and 8.7°C were recorded between October and June during 2007 and 2008 and long-term periods in winter sowing, respectively. Average temperatures of 14.8 and 13.8°C were recorded between March and June during 2007 - 2008 and long-term periods in spring sowing, respectively. Total precipitations of 252.0 and 359.1 mm were recorded between October and June during 2007 - 2008 and long-term periods in winter sowing, respectively. Total rainfalls of 148.3 and 199.0 mm were recorded between March and June during 2007 and long-term periods in spring sowing, respectively.

Eighteen grasspea lines (38, 439, 452, 455, 463, 481, 504, 508, 516, 519, 520, 522, 527, 528, 531, 553, 554 and 563) from ICARDA, five grasspea varieties (azureus, biflorus, coloratus, leucotetragonus and albus) from Romania, two grasspea populations from Adiyaman and Elazig provinces and one grasspea cultivar from Ankara Journal of Central Research Institute of Field (Gurbuz, 2001) were used. Seeds were sown on 31 October, 2007 and on 21 March, 2007 in Kazova-Tokat. Plot size was 6 x 1.8 = 10.8 m². Sowing rate of grasspea was 80 kg ha⁻¹. Fertilization were uniformly applied to the soil before sowing at a rate of 30 kg ha⁻¹ N and 80 kg ha⁻¹ P₂O₅ (Karadag and Buyukburc, 2004). Seeds were harvested at maturity of grasspea plots. A total of 25 g seed samples were selected randomly from each subplot representing each line for winter or spring sowing. The combined seeds were finely screened with 0.5 mm screen size hammer mill.

The grasspea seeds were analyzed using a reversed-phase

high-performance liquid chromatography method (RP HPLC) for α -ODAP and β -ODAP outlined by Wang et al. (2000). Reagents of α - and β -ODAP were purchased. Ultra-pure water was obtained by Milli-Q Millipore 18.2 MV cm⁻¹ resistivity. Acetonitrile (HPLC grade) and 1-fluoro-2,4-dinitrobenzene (FDNB) were purchased from Merck & Co.

The HPLC system consisted of a Perkin Elmer Model 200 series pump, a Dionex C₁₈ quard column 3.93 mm and C₁₈ 150 mm analytical column, a column heater and a Model 200 series dual wavelength absorbance detector set at 360 nm. Millennium 32 software from Perkin Elmer was used to control system operation and collect the data. Mobile phase A consisted of 0.05mol/l K₂HPO₄ and 1% dimethyl formamide in 24 water (v/v) and was adjusted to pH 5.60 with glacial acetic acid. Mobile phase B was acetonitrile. The mobile phase solutions were filtered through a 0.45 mm membrane filter and operated at a flow-rate of 1.0 ml/min. Sample preparations and precolumn derivatization procedures were adapted from Wang et al. (2000).

RESULTS AND DISCUSSION

Winter and spring sown twenty-six grasspea genotypes α , β and total ODAP contents are shown in Table 1. α -Oxalyldiaminopropionic acid (α -ODAP, 3-amino-2-oxalylaminopropanoic acid) is not toxic to animals. A ODAP levels of winter-sown grasspea genotypes varied between 49.31 to 419.74 mg kg⁻¹ for line 554 and Elazig, respectively. Overall average α -ODAP level of winter-sown grasspea genotypes was 187.83 mg kg⁻¹. α -ODAP levels of spring-sown grasspea genotypes ranged from 64.83 to 479.97 mg kg⁻¹ for line 504 and Adiyaman, respectively. Average α -ODAP level in spring-sown grasspea genotypes was 251.53 mg kg⁻¹. Since the level of α -ODAP is not related to toxicity, the variation in α -ODAP content may not be important in terms of forage crop quality (Bell, 2003).

β -ODAP is a close structural analogue of glutamic acid and acts as an agonist at nerve cells, which causes irreversible spastic paralysis of the legs known as neurolathyrism (Moges et al., 2004). β -ODAP levels in winter-sown grasspea genotypes changed from 27.60 to 1235.85 mg kg⁻¹ for line 531 and 516, respectively. Average of β -ODAP level in winter-sown grasspea genotypes was 459.62 mg kg⁻¹. Grasspea genotypes; 563, Elazig, 516, 531, 554, Adiyaman, 439, 452 and 522 had the lower level β -ODAP than overall average in winter-sown grasspea genotypes. β -ODAP levels in spring-sown grasspea genotypes were between 68.97 to 1028.01 mg kg⁻¹ for genotype Biflorus and 531, respectively. The average β -ODAP level in spring-sown grasspea genotypes was 540.88 mg kg⁻¹. Grasspea genotypes; Biflorus, 553, 527, 504, 463, 452, Elazig, 519, 508, 38, 520, 563, Coloratus and Leucotetragonus had the lower level β -ODAP than the average value in spring-sown grasspea genotypes. Grasspea genotypes; 452, 519, 508 and 527 had the lower β -ODAP level as compared to the average β -ODAP levels in winter- and spring-sown grasspea genotypes. Although ODAP level in spring-sown grasspea

Table 1. The α , β and total ODAP contents (mg kg^{-1}) of winter- and spring-sown grasspea genotypes.

Genotypes	Winter-sown grasspea			Spring- sown grasspea		
	α -ODAP	β -ODAP	Total ODAP	α -ODAP	β -ODAP	Total ODAP
38	155.96	532.52	668.48	157.58	434.77	592.35
439	63.39	270.67	334.06	324.78	617.62	942.40
452	112.47	271.73	384.20	76.01	272.09	348.10
455	157.83	368.31	526.14	330.27	780.79	1111.06
463	156.10	471.36	627.46	77.59	222.41	300.00
481	169.58	468.89	638.47	222.67	553.99	776.66
504	158.45	555.47	713.92	64.83	162.02	226.85
508	146.34	396.16	542.50	139.99	373.81	513.80
516	253.38	1235.85	1489.23	194.54	564.97	759.51
519	148.81	349.73	498.54	116.76	304.32	421.08
520	249.26	483.00	732.26	167.00	470.51	637.51
522	115.58	292.89	408.47	369.05	1002.59	1371.64
527	121.36	415.07	536.43	138.69	133.83	272.52
528	164.05	455.12	619.17	408.19	992.15	1400.34
531	94.07	27.60	121.67	354.47	1028.01	1382.48
553	354.56	505.55	860.11	99.95	111.84	211.79
554	49.31	136.05	185.36	343.23	899.17	1242.40
563	364.08	849.75	1213.83	361.51	471.46	832.97
Albus	139.37	349.08	488.45	351.48	778.42	1129.90
Azureus	158.28	372.35	530.63	431.88	896.96	1328.84
Biflorus	149.91	484.40	634.31	67.63	68.97	136.60
Coloratus	169.76	518.14	687.90	298.55	522.03	820.58
Leucotetragonus	258.23	474.83	733.06	266.06	538.15	804.21
Gurbuz 2001	385.95	559.12	945.07	421.21	881.49	1302.70
Adiyaman	167.71	204.74	372.45	479.97	698.91	1178.88
Elazig	419.74	901.83	1321.57	275.90	281.52	557.42
Mean	187.83	459.62	647.45	251.53	540.88	592.35

genotypes was higher than those of winter-sown ones (Table 1), it is not possible to conclude that winter or spring sown grasspea genotypes had higher β -ODAP levels due to the differences in influence of sowing time on β -ODAP. Winter-sown 531 line seeds had the lowest 27.60 mg kg^{-1} β -ODAP level among the all genotypes evaluated. The grasspea lines grown in spring contained 37.25 times greater β -ODAP level than those in winter-sown. The variation should be minimized in the seeds selected. β -ODAP levels of 481, Leucotetragonus, Coloratus, 452, 520, 508, 519, 38 grasspea genotypes had lower variations between spring and winter sowing.

Total ODAP levels of winter-sown grasspea genotypes ranged from 121.67 to $1489.23 \text{ mg kg}^{-1}$. The average β -ODAP levels of winter-sown grasspea genotypes were $645.07 \text{ mg kg}^{-1}$. Total ODAP levels of spring-sown grasspea genotypes varied between 136.60 to $1400.34 \text{ mg kg}^{-1}$. The average β -ODAP level of spring-sown grasspea genotypes was $592.35 \text{ mg kg}^{-1}$. Since total ODAP values are determined as sum of α -ODAP and β -

ODAP levels, it does not necessarily indicate the toxicity. However, total ODAP levels were reported to enable the comparison with some of the results obtained in other studies. The ODAP levels of grasspea genotypes were found between 0.01 and 7.20 g kg^{-1} (Campbell, 1997).

L. sativus is an important feed source, but the use of grasspea is affected by toxic β -ODAP levels. It is important for researchers to identify the exact β -ODAP content of grasspea varieties. The present study indicated a high variability in β -ODAP levels in winter- and spring-sown grasspea genotypes. Grasspea 452, 508 and 519 genotypes had lower β -ODAP levels and showed less variability between winter and spring sowings.

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