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Genetic affinity, ß –ODAP, homoarginine and asparagine contents of Turkish grass pea landraces

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Abstract. In 12 Turkish provinces grass pea has been cultivated for a long time. Throughout these provinces 52 grass pea landraces seeds were collected from 52 different districts. Genetic affinity levels among and inside the genotypes were determined by ISSR method. The data obtained from genetic analysis were processed by using NTSYSpc and a dendrogram has been generated. Similarity coefficients among the genotypes ranged from 0.43 to 0.82. ß-3-(-N-oxalyl)-L-2, 3-diamino propionik asit (ß –ODAP), homoarginine and asparagine analysis were done in grass pea seeds also, by using capillary electrophoresis. The data of this work reflects that ß –ODAP, homoarginine and asparagine contents of grass pea local landraces vary in a wide range from very low to high levels. A positive correlation between homoarginine and ß –ODAP quantities in seeds of 52 *Lathyrus* local landraces was proven in this work. Also, a correlation between asparagine and ß –ODAP levels was observed in some accessions. Because of grass pea genotypes with higher yield and low ß –ODAP content are relevant for future genetic and breeding studies, all of these accessions should be preserved as a genetic material.

Key words. Grass pea – Turkey - Genetic affinity – ß –ODAP – Homoarginine – Asparagine.

Affinité génétique, teneurs en ß-ODAP, homoarginine et asparagine des variétés locales de gesse de Turquie

Résumé. *Dans 12 provinces de Turquie, la gesse est cultivée depuis longue date. Dans ces provinces, des semences de gesse de 52 variétés locales ont été cueillies dans 52 districts différents. Les niveaux d'affinité génétique inter et intra-génotypes ont été déterminés par la méthode ISSR. Les données obtenues à partir de l'analyse génétique ont été traitées par NTSYSpc et un dendrogramme a été créé. Les coefficients de similarité entre les génotypes allaient de 0,43 à 0,82. Des analyses de -3-(-N-oxalyl)-L-2,3-diaminopropionic acid (ß-ODAP), homoarginine et asparagine ont été effectuées également sur les semences de gesse, par électrophorèse capillaire. Les données de ce travail reflètent que les teneurs en ß-ODAP, homoarginine et asparagine des variétés locales de gesse varient dans un large intervalle, depuis des niveaux très faibles jusqu'à des niveaux élevés. Une corrélation positive entre les quantités d'homoarginine et de ß-ODAP dans les semences des 52 variétés locales de* Lathyrus *a été trouvée dans ce travail. Une corrélation entre les niveaux d'asparagine et de ß-ODAP a également été observée dans certaines accessions. Étant donné que les génotypes de gesse présentant un meilleur rendement et une faible teneur en ß-ODAP sont importants pour de futures études de génétique et d'amélioration, toutes ces accessions devraient être conservées comme matériel génétique.*

Mots-clés. *Gesse – Turquie – Affinité génétique – ß-ODAP – Homoarginine – Asparagine.*

I – Introduction

Grass pea (*Lathyrus sativus* L.) has an amazing capability to survive under harsh environmental conditions (Campbell, 1997). Grass pea seeds have high nutritional value, with 26-30% protein content (Hanbury *et al.*, 2000). However widespread and abundant production and usage of *L. sativus* seeds as a food legume in human and animal diet has been prevented due to the pres-

ence of a free amino acid with neurotoxic effects: ß- N-*oxalyl*-*L*-α,ß-diaminopropionic acid (ß –ODAP) is suggested to be the causative agent for a paralysis of the lower limbs, known as "neurolathyrism". In addition to ODAP, *L. sativus* contains other free amino acids, such as homoarginine, which also play an important role in physiological and biochemical processes. There are controversial reports about the effects of homoarginine. Homoarginine has been proposed to modulate the toxicity of ß –ODAP (Shamin *et al.*, 2002).

Results have been successful in many studies in order to determine the phylogenetic relationships between and within *Lathyrus* populations using the ISSR primers. The aim of this paper was to provide information on the ß –ODAP, homoarginine and asparagine contents and genetic affinity of grass pea populations cultivated in Turkey.

II – Materials and methods

From various regions of Turkey, 51 grass pea and one *Lathyrus clymenum* local landraces were collected and cultivated in the experimental fields of Ondokuz Mayis University. One released cultivar was also included in the research. The seeds harvested in the year of 2011 were analysed for ß –ODAP, homoarginine and asparagine by a new, validated and simple capillary electrophoretic method. Calibration graphs were prepared by using standard mixtures. ODAP and amino acids were extracted from dry powdered grass pea seeds (0.50 g) by ethanol-water (30:70, v/v, 50.0 mL) mixture (Onar *et al.*, 2014). DNA analysis was performed in 5 plant stools by using appropriate primers and the method of ISSR to examine the genetic proximity.

III – Results and discussion

The most abundant of the three measured amino acids was homoarginine (Fig. 1). The concentration of homoarginine ranged from 2.14 mg/g to 1.27 mg/g (w/w). This amino acid was followed by ß –ODAP and the concentration ranged from 1.04 mg/g to 8.68 mg/g (w/w). For asparagine, the minimum concentration was 0.06 mg/g (w/w) while the maximum was 4.74 mg/g (w/w) of seed. Polignano *et al.* (2005) reported ß –ODAP grass pea seed concentrations as 0.24-0.64% in 47 progenies. Fikre *et al.* (2008) indicated that homoarginine was up to 0.8%, ß –ODAP was between 0.02% and 0.54% while asparagine content was between 0.01% and O.06% in 9 genotypes.

Comparison of these results with the data of this work reveals that homoarginine, ß –ODAP and asparagine concentrations of 52 *Lathyrus* local landraces seeds spread from low to high levels. Linear regression model of ANOVA revealed a positive significant correlation between ß –ODAP and homoarginine concentrations of seeds (Fig. 1). This is very important because homoarginine can modulate the toxicity of ß –ODAP (Shamin *et al.*, 2002).

This finding is in agreement with literature information of Piergiovanni and Damascelli (2011). Although there is a significant positive correlation between ß –ODAP and asparagine concentrations for some genotypes as can be seen in Fig. 1.

The ß –ODAP content of grass pea is known to vary widely, both among genotypes and environments (Campbell, 1997). We compared the data of ß –ODAP with the data published by Basaran *et al.* (2011) that belongs to the same *Lathyrus* local landraces (used the same analysis method). A statistical difference between ß –ODAP contents of 2011 harvest and collected samples in 2007 was found at 95% confidence level. The ß –ODAP data of this work reflect the differences due to the environment, because grass pea seeds of this study were obtained by cultivation of samples collected by Basaran *et al.* (2011).

The concentrations of the isolated DNA samples ranged between 17.3 nanogram/milliliter (ng/mL) and 267.5 ng/mL. In the used primers, the band sizes in each genotype were obtained ranging

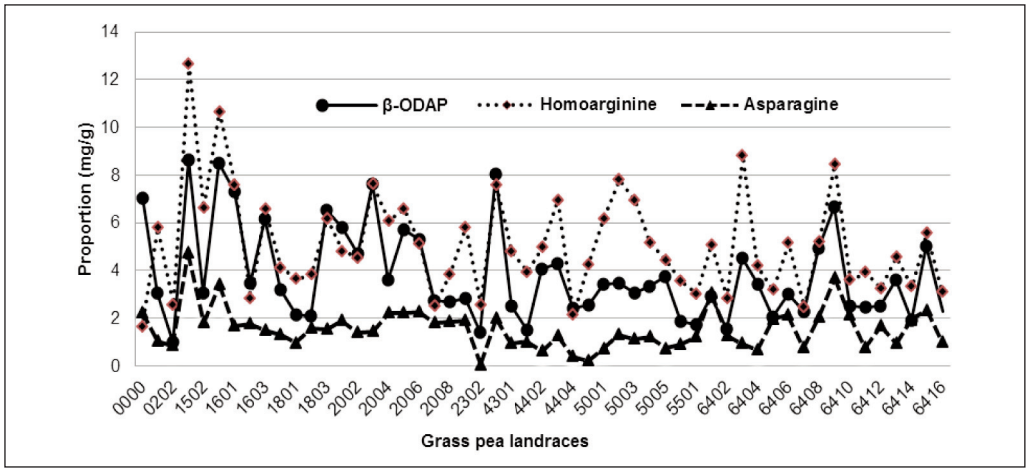

Fig. 1. Proportions of ß –ODAP, homoarginine and asparagine in seed extracts of 52 grass pea populations.

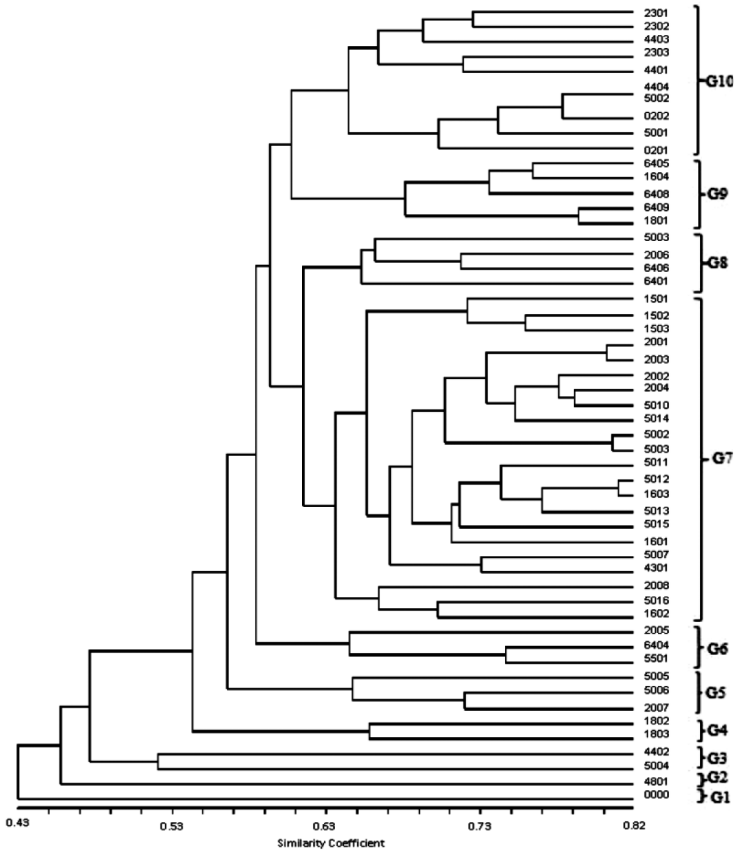

Fig. 2. Genetically similarity dendrogram created with ISSR markers for grass pea genotypes.

between 0.23 kilo base (kb) and 1.28 kb. The DNA samples from grass pea genotypes were analysed through the program of NTSYSpc 2.0 after screening and scoring via ISSR primers. The lowest genetic relationship was determined between the genotype numbered 4402 and cultivar Gurbuz with the value of 0.244. The maximum genetic relationship was determined between the genotype numbered 4404 and the genotype numbered 5002 with the value of 0.825. Furthermore, the analyses except cultivar Gurbuz showed that genetically the furthest genotypes were found to be the genotypes numbered 1802 and 5004 with the similarity coefficient of 0.313. The average values of genetic distances among all genotypes identified as 0.592. Ten main groups were formed in dendrogram (Fig. 2). The genotypes collected from similar ecological conditions have been found to combine as the basis of the subgroup of genotypes. Probably there has been seed exchange among the local farmers.

IV – Conclusions

The *Lathyrus* local landraces were collected from traditional farmers in various regions of Turkey. The data of this work reflects that ß –ODAP, homoarginine, asparagine contents and genetic affinity of grass pea local landraces vary in a wide range from very low to high levels. Although all of these accessions should be preserved as a genetic material, the grass pea genotypes with higher yield and low ß –ODAP content are relevant for future genetic and breeding studies.

A positive correlation between homoarginine and ß –ODAP quantities in seeds of 52 *Lathyrus* local landraces was proven in this work. This is very important because homoarginine can modulate the toxicity of ß –ODAP (Shamin *et al.*, 2002). The results obtained from this work and the other study conducted with the same landraces showed that environment highly affected ß –ODAP content of *Lathyrus* seeds.

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References

Basaran U., Asci O.O., Mut H., Acar Z. and Ayan I., 2011. Some quality traits and neurotoxin b-N-oxalyl-L-cz, diaminopropionic acid (ß –ODAP) contents of *Lathyrus sp.* cultivated in Turkey. *African journal of Biotechnology,* I0(20), p. 4072-4080.

Campbell C.G., 1997. *Grass pea, Lathyrus sativus L. promoting the conservation and use of underutilized and neglected crops. Nr I8.* Rome, Italy: Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany international Plant Genetic Resources Institute, p. 92.

Fikre A., Korbu L., Kuo Y. H. and Lambein F., 2008. The contents of the neuroexcitatory amino acid ß –ODAP. *Food Chemistry,* 110, p. 422-427.

Hanbury C.D., White C. L., MuHan B.P. and Siddique K.H.M., 2000. A review of the potential of *Lathyrus sativus* L. and *L. cicera* L grain for use as animal feed. *Animal Feed Science and Technology,* 87, p. 1-27.

Onar A.N., Erdoğan B.Y., Ayan I. and Acar Z., 2014. Homoarginine, ß –ODAP and asparagine contents of grass pea landraces cultivated in Turkey. *Food Chemistry*, 143 (2014), p. 277-281.

Polignano G.B., Uggenti P., Alba V., Bisignano V. and Della Gatta. C., 2005. Morpho-agronomic diversity in grasspea *(Lathyrus sativus* L.). *Plant Genetic Resources,* 3, p. 29-34.

Piergiovanni A.R. and Damascelli A., 2011. L-Homoarginine accumulation in grass pea *(Lathyrus sativus* L.) dry seeds. A preliminary survey. *Food and Nutrition Sciences,* 2, p. 207-213.

Shamin M.Z., Hossain M. S., Islam K., Yusuf H.K.M. and Lambein F., 2002. Mechanism of ODAP toxicity in one-day-old chicks. *Dhaka University journal of Biology Science,* 11 (1), p. 1-7.