ISSN 1392-3196 / e-ISSN 2335-8947
Zemdirbyste-Agriculture, vol. 109, No. 1 (2022), p. 35–42
DOI 10.13080/z-a.2022.109.005

Variations of genotypes of *Vicia* species as influenced by seed phenolic compounds and antioxidant activity

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Abstract

The phenolic compounds and antioxidant activity of different *Vicia* species, which are cultivated in different areas of Turkey, and the diversity between analysed characters were investigated. For this purpose, 9 genotypes from three *Vicia* species: common vetch (*Vicia sativa* L.), Hungarian vetch (*Vicia pannonica* Crantz.) and Narbon vetch (*Vicia narbonensis* L.), were used. The experiment was carried out to determine total phenolic content (TPC), content of individual phenolic compounds, to determine $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ scavenging activity and ferric-reducing antioxidant power (FRAP) in methanol and acetone extracts of seeds. The TPC of vetch extracts was in range 11.18–30.42 mg GAE g^{-1} (in methanol extract) and 17.05–59.88 mg GAE g^{-1} (in acetone extract). Two *V. sativa* genotypes Cvoe and Cvke stood out among the others with regard to high TPC and antioxidant activity. They also had the highest content of individual hydroxybenzoic acids and flavones. All extracts of *V. narbonensis* genotypes were characterised by absence of flavones and low TPC and antioxidant activity. GGE biplot analysis revealed the differences of *Vicia* genotypes based on phenolic compounds and antioxidant activity. The significant correlations among TEAC, FRAP and $DPPH^{\bullet}$ scavenging activity and the content of hydroxybenzoic acids were found in both methanol and acetone extracts ($P < 0.01$). The genotypes were divided into three clusters in acetone extract and two clusters in methanol extract with similarity above 60% in each group by a hierarchical cluster analysis.
These results demonstrated that the genotypic differences of *Vicia* species in terms of TPC and antioxidant activity can be a tool for feed technology studies for animal nutrition, animal welfare and meat quality.

Keywords: antioxidant activity, cluster analysis, GGE biplot, phenolic compounds, *Vicia* genotypes.

Introduction

Legume plants are of great economic importance and can be used in human nutrition, herbal medicine, as a source of oil and as animal feed (Koçak et al., 2011). The genus *Vicia* comprises approximately 190 species in the world, and approximately 66 species, 27 subspecies and 29 cultivars have been identified in Turkey (Başbağ et al., 2013). In most of the Mediterranean countries, including Turkey, vetches are the most common forage crops with the largest annual production (Tenikecier et al., 2017). Common vetch (*Vicia sativa* L.) is a winter crop mainly grown in Turkey, and cultivated cultivars can be used for high-quality animal feed intensively (Orak, Nizam, 2004). Hungarian vetch (*Vicia pannonica* Crantz) is a valuable crop in both Turkey and Central European countries with a high ability to adapt to the climatic and edaphic conditions. Because of its cold tolerance, it is planted in autumn in the Central Turkey, and has great potential for hay, straw and seed yield (Fırıncıoğlu et al., 1997). Narbon vetch (*Vicia narbonensis* L.), which has been cultivated for many years in Turkey as an annual legume species tolerant to cold and drought stresses, is readily found among native flora of Turkey (Orak, Nizam, 2004; Fırıncıoğlu et al., 2012).

Phenolic compounds are a large group of secondary plant metabolites, which have a variety of biological activities. Their presence in food and feed has several positive effects on human and animal health (Bravo, 1998; Zloch et al., 2018). In modern animal

Please use the following format when citing the article:
Orak H. H., Karamać M., Amarowicz R., Orak A., Janiak M. A., Tenikecier H. S. 2022. Variations of genotypes of *Vicia* species as influenced by seed phenolic compounds and antioxidant activity. Zemdirbyste-Agriculture, 109 (1): 35–42. DOI 10.13080/z-a.2022.109.005

nutrition, these natural antioxidants are an alternative to synthetic feed additives that have been controversial, in recent years, in terms of animal welfare and the quality of animal products. Phenolic compounds inhibit growth of pathogenic bacteria in the intestinal tract while allowing the development of beneficial bacteria (Brenes et al., 2016). Phenolic compounds are gaining importance for improving animal health due to their anti-inflammatory and antimicrobial effects. In a recent publication, Mahfuz et al. (2021) concluded that the supplementation of phenolic compounds as natural feed additives may have a role on the antioxidant, immunity, antimicrobial and overall production performance in poultry and swine. Their positive effect on gut can improve nutrient absorption in animals (Kamboh et al., 2015).

Dietary supplementation with rich source of phenolic compounds decreased some pathogenic bacteria such as *Clostridium* and *Enterococcus* spp. in animals (Leusink et al., 2010). Therefore, antioxidants have been used in commercial feeds to prevent lipid peroxidation and oxidative rancidity of feeds (Decker et al., 2012). Salami et al. (2016) suggested that there should be careful consideration for the use of new antioxidant substances, particularly plant extracts, with respect to their effect on meat quality, improvement in meat colour and lipid oxidative stability.

A recently reported study showed that the antioxidant capacity of *V. sativa* is comparable with that of soybean (Megías et al., 2009). Strong antioxidant activity of *V. sativa* has also been determined by Amarowicz et al. (2008). A screening for antioxidant activity of *Vicia* 28 species located in Spain has been reported by Pastor-Cavada et al. (2011), and the highest TPC was found in *V. sativa*. Salehi et al. (2021) reported that *Vicia* plants were evaluated in the usual dietary habits regarding potential health beneficial properties, when safety concerns and therapeutic or preventive efficacy should be carefully evaluated. However, according to our knowledge, research to date regarding the profile of phenolic compounds of vetch seeds and its effect on the antioxidant capacity of seeds is limited.

Therefore, the aim of this study was to determine the composition of phenolic compounds and the antioxidant activity of methanol and acetone extracts from the seeds of 9 *Vicia* genotypes belonging to *V. pannonica*, *V. narbonensis* and *V. sativa* species. These genotypes are representative of the variability of the genus *Vicia* in Turkey. To the best of our knowledge, the present research discloses the first report on phenolic compounds and antioxidant activity of *V. pannonica V. narbonensis* and *V. sativa* in methanol and acetone extracts.

Materials and methods

The plant material used in the experiment was obtained from the Field Crops Department, Agricultural Faculty of Namik Kemal University in Turkey in 2013, which resulted from continuous variety improvement studies. Seeds were selected from vetches with high forage and seed yields that were kept as genetic material. The seeds of 9 *Vicia* genotypes selected for further laboratory analyses were stored in paper bags under dry conditions at a temperature of +5°C. Two of them, referred to as 42.1 (Hv42.1) and Ege Beyazı (Hveb), belong to Hungarian vetch (*V. pannonica* Crantz.) species; genotypes Ege KF (Nvek), Pop KF (Nvt) and HSN 908 (Nv 908) are known as Narbon vetch (*V. narbonensis* L.); Selçuk 99 (Cvse), Orakefe (Cvoe), Karaelçi (Cvke) and Tekirdağ Pop (Cvt) belong to common vetch (*V. sativa* L.) species. Lyophilised extracts obtained from seeds were used for further antioxidant activity analysis and investigation of individual phenolic compounds. Preparation of extracts, analysis of antioxidant activity and investigation of individual phenolic compounds were carried out at the Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences in Poland between 2013–2018. Laboratory experiment was carried out according to a randomized complete block design with three replications.

Preparation of extracts. After milling, dried seeds were extracted using 80% (v/v) methanol or 80% (v/v) acetone. Sample: solvent ratio was 1:10 (v/w). Suspensions were shaken in a shaking water bath SW22 (Julabo, Germany) at 70°C temperature for 15 min (Karamać et al., 2018). The extractions were repeated three times, and solvents were removed under vacuum using a rotavapor R-200 (Büchi Labortechnik, Switzerland). Before analysis, samples were lyophilisated with a Labconco freeze dryer Lyph Lock 6 freeze-dry system (Labconco, USA). The yield of extract (extractable components) expressed on dry weight basis of seed was calculated using the following equation:

Percentage extract yield (g 100 g^{-1}) = (dry extract weight / dry starting material weight) × 100,

where dry extract weight is the weight of the extract residue obtained after solvent removal.

Total phenolic content (TPC) of vetch seed extracts was determined by using Folin-Ciocalteu's reagent at 725 nm with a spectrophotometer DU-7500 (Beckman Instruments, USA) (Amarowicz et al., 2004). The TPC values were expressed as mg of gallic acid equivalent (GAE) per g of extract.

Trolox equivalent antioxidant capacity (TEAC). To determine TEAC, assay with 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical cation ($ABTS^{\bullet+}$) was conducted. $ABTS^{\bullet+}$ was generated and reaction was performed according to Re et al. (1999) description, and measurements were done at 734 nm. Results of $ABTS^{\bullet+}$ assay were expressed as mmol Trolox equivalents (TE) per g of extract.

The 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) scavenging activity of extracts was determined according to the method of Brand-Williams et al. (1995). Briefly, 2 mL of methanol was mixed with solution of $DPPH^{\bullet}$ (0.25 mL, 1 mM) and extract in methanol (0.1 mL, 0.5–2.5 mg mL^{-1}). Samples were placed in the dark for 20 min for reaction, and the absorbance was measured at 517 nm. Extract concentration needed to scavenge 50% of the initial $DPPH^{\bullet}$ (EC_{50}) values was obtained based on the plots of percentage of $DPPH^{\bullet}$ inhibition *vs* extract concentration.

Ferric-reducing antioxidant power (FRAP) of vetch extracts assayed as previously described by Benzie and Strain (1996). FRAP values were expressed as µmol Fe^{2+} equivalents per g of extract by using the calibration curve for ferrous sulphate ($FeSO_4$).

Phenolic compounds analysis. The separation and determination of phenolic compounds was performed using a Shimadzu HPLC system (Japan) consisting of two LC-30AD pumps, a CBM-20A system controller, a DGU-20A5R degassing unit, an SIL-30AC autosampler and an SPD-M30A diode array detector (DAD). The system was connected to a Luna C8(2) column, 3 µm, 150 × 4.6 mm (Phenomenex, USA). Mobile phase included acetonitrile-water-trifluoroacetic acid (TFA) (5:94.9:0.1, v/v/v) (solvent A) and acetonitrile-TFA (99.9:0.1, v/v)

(solvent B) (Orak et al., 2018). Flow rate of mobile phase was 1 mL min^{-1} in gradient system from 0% to 40% of solvent B from 0–18 min. Volume of injection of methanolic solution of extract (20 mg mL^{-1}) was 10 µL. The DAD was scanned over a wavelength range of 200 to 400 nm. The quantification of individual compounds numbered 1–10 (C1–C10) was based on calibration curves of corresponding standards.

Statistical analysis was carried out in Field Crops Department, Agricultural Faculty of Namik Kemal University. At least three replications were conducted for each analysis. The analysis of variance (one-way ANOVA) was performed by using statistical package of software package *MSTAT-C* (Michigan State University, USA) followed by the least significant difference (LSD) test. Differences were considered to be significant at $P < 0.05$. Pearson's test and Ward's method were applied to correlation and hierarchical cluster analysis of data, respectively. To show genotype by trait (GT) two-way data in genotype by environment (GGE) biplot analysis, the GT biplot model was used (Yan, Rajcan, 2002) All GT biplots were done using software package *GGE biplot* (Yan et al., 2007). To give an overview of the influence of genotypes on analysed characters, genotypes were compared by GGE biplot analysis and were also used to determine relationships between the phenolic profile and antioxidant activity. The relationships were compared by using Pearson's correlation coefficient (*r*).

Results and discussion

Extraction yield and total phenolic content (TPC). The extraction yield of *Vicia* genotypes ranged from 7.17 (Nv908) to 9.14 (Cvoe) g 100 g^{-1} in methanol extract and from 2.91 (Nvt) to 6.38 (Hv42.1) g 100 g^{-1} in acetone extract (Table 1). The TPC of vetch seeds in methanol extract ranged between 11.18 mg GAE g^{-1} (Nvt) and 30.42 mg GAE g^{-1} (Cvke). In acetone extract, the highest TPC (59.88 mg GAE g^{-1}) was determined for Cvoe, and the high TPC was determined also in acetone extract for Cvke and Hv42.1 (Table 1).

The results show that *V. narbonensis* genotypes had lower TPC compared to ones of *V. sativa* and *V. pannonica.* Moreover, *V. sativa* genotypes had the highest TPC of the three species. According to solvent efficiency, the acetone extraction gave higher TPC than methanol extraction for vetch samples. Results obtained in our experiment were in line with the literature data: Pastor-Cavada et al. (2011) determined that the *V. sativa* had the highest polyphenol content of all 28 *Vicia* species analysed.

Antioxidant activity. The $ABTS^{\bullet+}$ assay of extracts was reported in terms of TEAC in Table 1. The results of the $ABTS^{\bullet+}$ assay reveal that the *V. sativa* genotypes had higher activity than ones of *V. narbonensis* and *V. pannonica*. The highest TEAC was determined in both methanol and acetone extracts of genotype Cvoe. The lower TEAC was revealed in genotypes of *V. narbonensis*, i.e., 0.10 mmol TE g^{-1} for Nvek (in methanol extract) and 0.18 mmol TE g^{-1} for Nv908 and Nvek (in acetone extract). The TEAC of vetch seeds according to species decreased in the order: *V. sativa* > *V. narbonensis* > *V. pannonica*.

FRAP values ranged from 41.0 µmol Fe^{2+} g^{-1} (Nvek) to 291.4 µmol Fe^{2+} g^{-1} (Cvoe) in methanol extract and from 148.3 µmol Fe^{2+} g^{-1} (Nvt) to 611.2 µmol Fe^{2+} g^{-1} (Cvoe) in acetone extract. In general, genotypes of *V. sativa* exhibited higher antioxidant activity in $ABTS^{\bullet+}$ and FRAP assays (except FRAP of Hv42.1) similar to TPC. This remark is in line with literature data: Pastor-Cava et al. (2011) determined higher antioxidant activity in methanol extract of *V. sativa* compared to that of *V. narbonensis* and 26 other *Vicia* species.

The $DPPH^{\bullet}$ scavenging activity of acetone extract was higher than that of methanol extract (Figure 1). The effective concentration to cause 50% inhibitions (EC_{50} values) of $DPPH^{\bullet}$ determined in acetone extract ranged from 0.05 mg mL^{-1} (Cvoe) to 0.37 mg mL^{-1} (Cvoe) (Figure 2). In methanol extract, EC_{50} values were between 0.26 mg mL^{-1} (Cvoe) to 0.80 mg mL^{-1} (Nv 908).

Phenolic compound content. The results of quantitative analysis of phenolic compounds are presented in Table 2. Compounds 1, 2, 4 and 6 were

Table 1. Extract yield, total phenolic content (TCP), Trolox equivalent antioxidant capacity (TEAC) and ferric-reducing antioxidant power (FRAP) in methanol (MeOH) and acetone (ACET) extracts of seeds of 9 *Vicia* genotypes

Genotype	Extract yield, g extract 100 g^{-1}		TPC mg GAE g^{-1}		TEAC mmol TE g^{-1}		FRAP µmol Fe^{2+} g^{-1}	
	MeOH	ACET	MeOH	ACET	MeOH	ACET	MeOH	ACET
Hv42.1 *V. pannonica* Crantz.	8.58	6.38	15.75 ± 0.80 f	41.63 ± 3.08 c	0.12 ± 0.01 f	0.46 ± 0.02 e	107.9 ± 3.5 d	403.5 ± 2.3 d
Hveb *V. pannonica* Crantz.	8.73	3.81	17.96 ± 1.33 e	23.54 ± 0.04 f	0.11 ± 0.01 g	0.24 ± 0.01 f	77.4 ± 4.7 f	292.4 ± 4.0 e
Nvek *V. narbonensis* L.	8.20	3.08	13.90 ± 0.06 g	17.73 ± 0.37 g	0.10 ± 0.02 h	0.18 ± 0.01 h	41.0 ± 5.0 i	173.4 ± 4.5 g
Nvt *V. narbonensis* L.	8.86	2.91	11.18 ± 0.43 h	17.05 ± 1.13 g	0.11 ± 0.01 g	0.19 ± 0.02 g	62.3 ± 4.2 g	148.3 ± 3.8 i
Nv 908 *V. narbonensis* L.	7.17	3.75	13.24 ± 0.26 g	17.57 ± 1.00 g	0.13 ± 0.02 e	0.18 ± 0.02 h	51.3 ± 3.5 h	163.5 ± 15.4 h
Cvse *V. sativa* L.	8.08	6.22	23.42 ± 2.06 c	37.47 ± 1.38 d	0.36 ± 0.01 d	0.49 ± 0.02 b	138.4 ± 4.9 c	395.4 ± 3.5 c
Cvoe *V. sativa* L.	9.14	5.45	29.17 ± 1.01 b	59.88 ± 2.15 a	0.45 ± 0.02 a	0.58 ± 0.01 a	291.4 ± 8.7 a	611.2 ± 6.7 a
Cvke *V. sativa* L.	7.57	5.83	30.42 ± 0.84 a	55.97 ± 2.75 b	0.36 ± 0.02 c	0.48 ± 0.04 c	197.4 ± 7.1 b	572.0 ± 8.2 b
Cvt *V. sativa* L.	9.04	4.13	21.06 ± 0.69 d	30.79 ± 0.74 e	0.37 ± 0.01 b	0.44 ± 0.03 d	94.5 ± 9.0 e	264.4 ± 11.3 f

Note. GAE – gallic acid equivalent, TE – Trolox equivalent; data are expressed as the mean ± standard deviation (n = 3) for extract of each genotype; values in the same column having different letters differ significantly at $P < 0.05$.

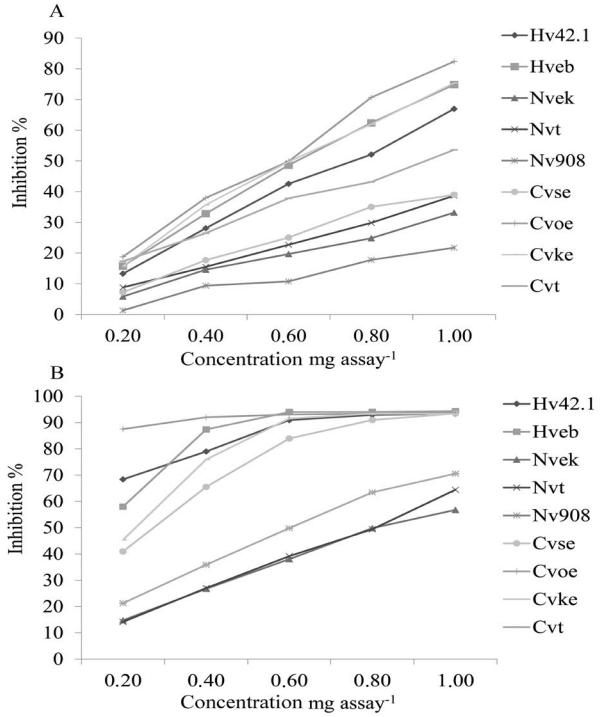
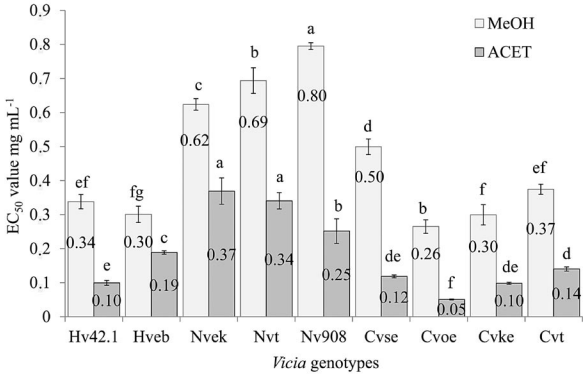


Figure 1. DPPH• scavenging activity of seeds of 9 *Vicia* genotypes in methanol (A) and acetone (B) extracts (n = 3)



Note. Data are expressed as the mean ± standard deviation (n = 3) for extract of each genotype; bars having different letters (separately for methanol and acetone extracts) differ significantly at $P < 0.05$.

Figure 2. EC_{50} values of DPPH• scavenging activity of seeds of 9 *Vicia* genotypes in methanol (MeOH) and acetone (ACET) extracts (n = 3)

tentatively identified as hydroxybenzoic acids on the base of the shape and maxima at 272–278 nm of UV-DAD spectra (Janiak et al., 2017). Their contents in extracts were expressed as GAE. These compounds are the major phenolics of the extracts. Compound 1 ranged from 0.33 mg g^{-1} (Nvt) to 5.31 mg g^{-1} (Cvoe) in methanol extract and from 0.68 mg g^{-1} (Nvek) to 7.85 mg g^{-1} (Cvoe) in acetone extract. The content of compounds 2 and 6 was highest in both methanol and acetone extracts in the Cvoe genotype. Compound 3 (C3) was identified as protocatechuic acid by comparison of retention time and spectral data with standard; it is next hydroxybenzoic acid present in seed. Protocatechuic acid was determined in all *V. narbonensis* and *V. sativa* genotypes in two extracts, although it was not found in one genotype Hveb in both extracts studied.

Table 2. Content (mg g^{-1}) of individual phenolic compounds (C1–C10) of seeds of 9 *Vicia* genotypes in methanol (MeOH) and acetone (ACET) extracts

Genotype	Extract	Phenolic compounds									
		C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
Hv 42.1	MeOH	1.17 ± 0.06 d	0.51 ± 0.02 e	0.09 ± 0.00 b	0.13 ± 0.0 b	0.04 ± 0.00 a	0.53 ± 0.03 e	nd	nd	nd	0.12 ± 0.05 a
Hveb	ACET	3.47 ± 0.16 c	1.33 ± 0.062 d	nd	nd	nd	0.98 ± 0.047 d	nd	0.49 ± 0.022 b	nd	0.14 ± 0.01 b
	MeOH	1.12 ± 0.05 d	0.67 ± 0.03 d	nd	0.13 ± 0.07 b	0.04 ± 0.00 a	0.39 ± 0.02 f	nd	nd	nd	0.12 ± 0.01 a
Nvek	ACET	1.52 ± 0.07 d	0.64 ± 0.03 e	nd	0.20 ± 0.01 b	nd	0.48 ± 0.02 e	nd	nd	nd	0.23 ± 0.10 a
	MeOH	0.39 ± 0.02 fg	0.23 ± 0.01 h	0.09 ± 0.00 c	0.37 ± 0.02 a	0.03 ± 0.00 ab	0.30 ± 0.01 g	0.04 ± 0.00 c	nd	nd	nd
Nvt	ACET	0.68 ± 0.03 f	0.35 ± 0.02 f	0.21 ± 0.01 c	nd	0.32 ± 0.01 a	0.32 ± 0.01 g	0.12 ± 0.01 a	nd	nd	nd
	MeOH	0.33 ± 0.02 g	0.40 ± 0.02 f	0.09 ± 0.00 a	0.35 ± 0.18 a	0.04 ± 0.02 a	0.31 ± 0.01 g	0.05 ± 0.00 a	nd	nd	nd
Nv 908	ACET	0.69 ± 0.03 f	0.32 ± 0.01 f	0.22 ± 0.01 a	0.85 ± 0.04 a	0.11 ± 0.05 b	0.38 ± 0.02 f	0.11 ± 0.01 b	nd	nd	0.02 ± 0.01 c
	MeOH	0.50 ± 0.02 f	0.32 ± 0.01 g	0.06 ± 0.00 e	0.28 ± 0.14 a	nd	0.23 ± 0.01 h	0.02 ± 0.00 e	nd	nd	nd
Cvse	ACET	0.73 ± 0.04 f	0.36 ± 0.02 f	0.21 ± 0.01 b	0.73 ± 0.37 a	0.09 ± 0.04 b	0.42 ± 0.02 f	0.09 ± 0.001 c	nd	nd	nd
	MeOH	4.12 ± 0.18 c	1.81 ± 0.07 c	0.06 ± 0.00 e	nd	nd	0.90 ± 0.04 b	nd	0.04 ± 0.00 d	0.03 ± 0.00 bc	nd
Cvoe	ACET	5.76 ± 0.26 b	2.43 ± 0.10 b	0.15 ± 0.01 d	0.06 ± 0.00 b	nd	1.34 ± 0.06 b	0.02 ± 0.00 f	0.20 ± 0.01 d	0.11 ± 0.05 d	nd
	MeOH	5.31 ± 0.25 a	2.50 ± 0.11 a	0.02 ± 0.00 f	0.04 ± 0.02 bc	nd	1.52 ± 0.07 a	0.02 ± 0.00 d	0.14 ± 0.01 b	0.08 ± 0.00 bc	nd
Cvke	ACET	7.85 ± 0.38 a	4.19 ± 0.19 a	nd	0.04 ± 0.02 b	nd	2.16 ± 0.10 a	0.06 ± 0.00 e	0.44 ± 0.02 c	0.19 ± 0.01 b	nd
	MeOH	4.48 ± 0.20 b	2.50 ± 0.11 a	0.05 ± 0.00 d	0.09 ± 0.00 bc	nd	0.79 ± 0.04 c	0.04 ± 0.00 b	0.86 ± 0.04 a	0.33 ± 0.17 a	nd
	ACET	5.87 ± 0.23 b	2.31 ± 0.10 c	0.11 ± 0.01 e	nd	nd	1.06 ± 0.05 c	0.09 ± 0.00 d	1.91 ± 0.09 a	0.65 ± 0.03 a	nd
CVt	MeOH	3.99 ± 0.18 b	2.05 ± 0.10 b	nd	0.03 ± 0.00 bc	0.03 ± 0.00 b	0.71 ± 0.03 d	0.01 ± 0.00 f	0.12 ± 0.01 c	0.11 ± 0.00 b	0.02 ± 0.00 b
	ACET	1.08 ± 0.05c	0.56 ± 0.02 e	0.09 ± 0.00 f	nd	nd	0.37 ± 0.02 f	nd	0.21 ± 0.01 d	0.14 ± 0.01 c	0.05 ± 0.00 c

Note. C1, C2, C4 and C6 were quantified on the basis of gallic acid, C3 – protocatechuic acid, C5 and C7 – quantified on the basis of *p*-coumaric acid, C8, C9 and C10 – quantified on the basis of apigenin; data are expressed as the mean ± standard deviation (n = 3); values in the same column having different letters differ significantly at $P < 0.05$; nd – not detected.

Compounds 5 and 7 were characterized by maximum absorption of UV-DAD spectra at 311–313 nm and shoulders at the short wavelength and were classified as hydroxycinnamic acids (Janiak et al., 2017). They were quantified on the basis of *p*-coumaric acid. Content of compound 5 was the highest in acetone extract of genotype Nvek, and it was not determined in any *V. sativa* species. Compound 7 was not found in *V. pannonica* species, although the highest content was in acetone extract of genotype Nvek, which is *V. narbonensis*. The presence of gallic and *p*-coumaric acids in *V. sativa* (cultivated), *V. narbonensis* (Bulgaria) and *V. pannonica* (cultivated) has been described in the literature (Malenčić et al., 2018). In turn, Magalhães et al. (2017) in seeds of *V. sativa* identified only two hydroxybenzoic acids, namely, protocatechuic and *p*-hydroxybenzoic. In our experiment, maximal absorption of UV-DAD spectra of compounds 8, 9 and 10 were at 267–271 and 333–

336 nm. Therefore, these compounds were qualified as flavones and quantified as apigenin equivalents. The compounds 8 and 9 were detected mainly in extracts of *V. sativa* species, although their content was definitely lower compared to that noted for phenolic acids. In previous study of Magalhães et al. (2017), the flavonoids were not determined in seeds of *V. sativa*.

Chemometric analysis. According to Pearson's correlation analysis, TPC strongly correlated with results of all antioxidant activity assays. The highest correlation was found with TEAC (r = 0.892**, P < 0.01) in methanol extract and with FRAP in acetone extract (r = 0.958**, P < 0.01). In the case of individual phenolic compounds, the content of dominant phenolics (C1, C2 and C6) strongly correlated (P < 0.01) with TEAC, FRAP and DPPH· scavenging activity in both methanol and acetone extracts. Content of compound 7 showed significant correlation with results of DPPH scavenging activity (r = −0.503**, P < 0.01) in acetone extract. The content of flavones (C8 and C9) significant correlated with TPC, TEAC, FRAP and DPPH· scavenging activity, but the correlations are not as strong as with some hydroxybenzoic acids (Table 3).

Table 3. Pearson's correlation coefficients (r) between total phenolic content (TPC), content of individual phenolic compounds (C1–C10) and antioxidant activity values of extracts of seeds of *Vicia* genotypes

	C1	C 2	C3	C4	C5	C6	C7	C8	C9	C10	DPPH· (EC_{50})	FRAP	TEAC	TPC
TPC	**0.939****	**0.948****	**−0.415***	**−0.675****	**−0.607****	**0.849****	**−0.032 ns**	**0.804****	**0.708****	**−0.194 ns**	**−0.718****	**0.884****	**0.892****	**1**
	0.927**	0.900**	−0.590**	−0.556**	−0.576**	0.861**	−0.229 ns	0.728**	0.702**	−0.143 ns	−0.865**	0.958**	0.910**	1
TEAC	**0.977****	**0.975****	**−0.468***	**−0.661****	**−0.605****	**0.886****	**−0.008 ns**	**0.683****	**0.595****	**−0.382***	**−0.553****	**0.828****	**1**	
	0.872**	0.855**	−0.554**	−0.588**	−0.643**	0.830**	−0.444*	0.513**	0.542**	−0.154 ns	−0.898**	0.856**	1	
FRAP	**0.852****	**0.836****	**−0.279 ns**	**−0.571****	**−0.564****	**0.949****	**0.023 ns**	**0.680****	**0.507****	**−0.180 ns**	**−0.659****	**1**		
	0.944**	0.925**	−0.553**	−0.531**	−0.554**	0.864**	−0.177 ns	0.703**	0.735**	−0.142 ns	0.820**	1		
DPPH· (EC_{50})	**−0.642****	**−0.643****	**0.516****	**0.651****	**−0.013 ns**	**−0.635****	**0.363 ns**	**−0.415***	**-0.439***	**−0.438***	**1**			
	−0.795**	−0.767**	0.734**	0.533**	0.818**	0.610**	−0.503**	−0.481*	−0.107 ns	−0.865**	1			

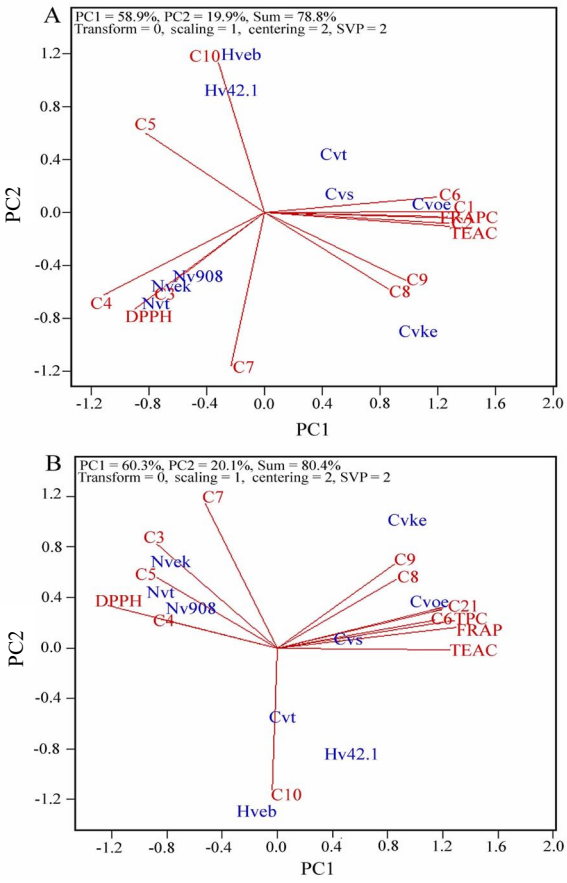
*, ** – significant at P < 0.05 and P < 0.01, ns – not significant; C1–C10 – explanation under Table 2; first line (bold lines) – correlations of methanol, second line – correlations of acetone extract data

The distribution and correlation analysis between the characters in both extracts according to GGE biplot is shown in Figure 3. GGE biplot analysis was also used to help interpret relationships between the phenolic profile and determined antioxidant activity values in *Vicia* genotypes. Close positive associations among C1, C2 and C6 (hydroxybenzoic acids), TPC, TEAC and FRAP were found. Another close positive association was determined between flavones C8 and C9. These compounds were accumulated in *V. sativa* species. C3 (protocatechuic acid) and C4 are closely associated with DPPH· scavenging activity both in methanol and acetone extracts.

The GGE biplot analysis had previously been used to show the effects of genotypes on phenolic compound distribution and antioxidant activity of 9 *Olea* genotypes; similarities and differentiations between genotypes were determined Orak et al. (2019). In our study, the "average tester coordination" view of the GGE biplot is presented in Figure 3A and B. These graphs visualise the interrelationship among characteristics. The close positive associations between TPC, FRAP, TEAC and C1, C6 are shown in methanol extract (Figure 3A). The same relations were determined between TPC, FRAP, TEAC and compound C1, C6 in acetone extract (Figure 3B).

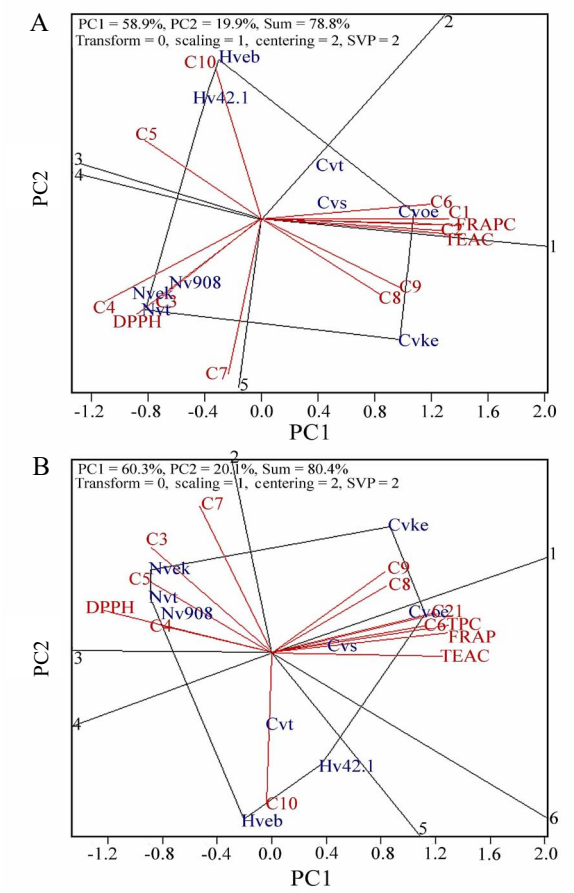
The GGE biplot "which-won-where/what" shows a polygon view with some genotypes as vertices (Yan, Rajcan 2002). Perpendicular lines are drawn for each side of the polygon, and the biplot was divided into sectors. The vertex genotypes are the most responsive and are either best or poorest for one or all characteristics in each sector. The data of profile of phenolic compounds and antioxidant activity values were subjected to GGE biplot analysis to show the differences or similarities between genotypes.

The vectors from the centre of the GGE biplot (0.0) divided the graph into 5 sectors with methanol extract (Figure 4A). In this polygon, 4 vertex genotypes Cvoe, Hveb, Nvt and Cvke were differentiated from the other ones with the longest distance from point of the



C1–C10 – explanation under Table 2; *Vicia* genotypes: Hv – *V. pannonica*, Nv – *V. narbonensis*, Cv – *V. sativa*

Figure 3. The distribution and correlation between the total phenolic content (TPC), content of individual phenolic compounds (C1–C10) and antioxidant activity values in the vector space according to genotype by environment (GGE) biplot analysis of seeds of 9 *Vicia* genotypes in methanol (A) and acetone (B) extracts



C1–C10 – explanation under Table 2; *Vicia* genotypes: Hv – *V. pannonica*, Nv – *V. narbonensis*, Cv – *V. sativa*

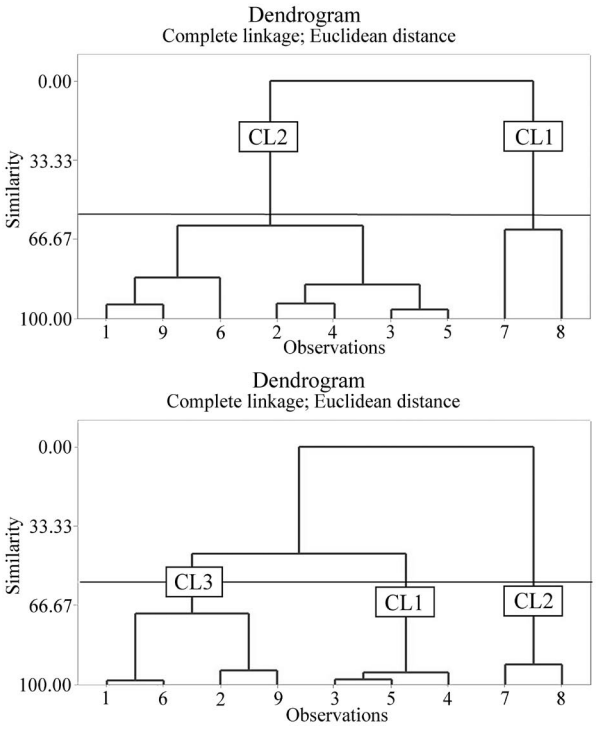
Figure 4. Genotype by environment (GGE) biplot polygon view of individual phenolic compounds (C1–C10) and antioxidant activity values of seeds of 9 *Vicia* genotypes in methanol (A) and acetone (B) extracts

origin of GGE biplot. In particular, the Cvoe was clearly distinct from the other genotypes, and it was characterised by C1, C2, C6, FRAP, TEAC and TPC. In turn, the Hveb differed from the other genotypes along with compounds C10 and C5. The genotype Nvt was at a short distance from protocatechuic acid (C3), compounds C4 and C7, and DPPH· scavenging activity. The last vertex genotype Cvke was close to flavones (C8 and C9).

In acetone extract, 6 vertex genotypes formed in the 6-sided polygon: the genotypes Cvoe, Cvke, Nvek, Nvt, Hveb and Hv42.1 were placed on the vertex positions (Figure 4B). Genotype Cvoe was close to C1, C2 and C6 (hydroxybenzoic acids), TPC, FRAP and TEAC. Cvke was clearly distinct from the other genotypes along with flavones (C8 and C9). On the other hand, Nvek was clearly distinct from the other genotypes with protocatechuic acid (C3), compounds C4 and C7, and DPPH· scavenging activity. The vertex genotypes Hveb and Hv42.1 differed from the other ones in content of compound C10. Compound C5 belonging to hydroxycinnamic acids differentiated methanol and acetone extracts. It was not connected with DPPH· scavenging activity in methanol extract, although it was related to it in acetone extract.

Cluster analysis was approved as a suitable method for analysing genetic relationships among samples (Mohammadi, Prasanna, 2003); therefore, to show the similarity and differences between genotypes a dendrogram was prepared (Figure 5). Nine *Vicia* genotypes were divided into two clusters at level of 60% similarity to each other with methanol extract.

This result shows that Cvoe and Cvke belonging to *V. sativa* genotypes differentiated all other ones, and they were placed in the 1st cluster (CL1) with regard to high TPC, FRAP, TEAC and DPPH· scavenging activity along with high content of compounds C1, C2 and flavones (Figure 5A). This can be explained by the fact that these highlighted compounds could be strongly related to the antioxidant activity values of these genotypes. The 2nd cluster (CL2) included the rest of the genotypes. In acetone extract, 9 *Vicia* genotypes were divided into tree clusters at level of 60% similarity (Figure 5B).



1 – Hv 42.1, 2 – Hveb, 3 – Nvek, 4 – Nvt, 5 – Nv 908, 6 – Cvse, 7 – Cvoe, 8 – Cvke, 9 – CVt

Figure 5. Cluster (CL) analysis (hierarchical method) of seeds f 9 *Vicia* genotypes in methanol (A) and acetone (B) extracts

Genotypes Nvek, Nvt and Nv908 belonging to *V. narbonensis* were in the 1st cluster. They were characterised by absence of flavones (C8, C9 and C10) and the lowest TPC, FRAP, TEAC and DPPH· scavenging activity. Genotypes Cvoe and Cvke were included in the 2nd cluster **(**CL2) with regard to high TPC, FRAP, TEAC, DPPH· scavenging activity and high content of compounds C1, C2 and flavones. The 3rd cluster (CL3) contains two genotypes Hv42.1 and Hveb of *V. pannonica* and two genotypes Cvse and Cvt of *V. sativa*. Genotypes Cvse and Cvt differentiated from the other two *V. sativa* ones in respect to antioxidant activity. The distribution of *V. sativa* genotypes between two CL2 and CL3 clusters may result from their different TPC and antioxidant activity values. Lower content of hydroxybenzoic acids of Cvse and Cvt put these genotypes closer to those of *V. pannonica*. Also, the antioxidant activity values of genotypes Cvse and Cvt was more similar to that of the *V. pannonica* genotypes Hv42.1 and Hveb than to other *V. sativa* genotypes Cvoe and Cvke (Figure 5B).

According to these results, it can be concluded that potential *Vicia* genotypes with high TPC and antioxidant activity values can be used to obtain higher quality animal products. Additionally, these results can be utilised for using *Vicia* genotypes containing high TPC and antioxidant activity for human nutrition after eliminating non-nutritional factors.

Conclusions

1. The effects of genotypes on the variations of phenolic compounds and antioxidant activity values were determined in this study. In order to determine the effect of genotype on the phenolic compound contents and antioxidant activity values in vetch seeds, three *Vicia* species: *V. pannonica*, *V. narbonensis* and *V. sativa*, were analysed. In general, *V. sativa* genotypes exhibited higher total phenolic content (TPC) and antioxidant activity values than others. The acetone extraction gave higher TPC than methanol extraction according to solvent efficiency.

2. The profile of phenolic compounds of 9 *Vicia* genotypes was determined. In total, up to 9 phenolic compounds were found. Although the content of flavones compounds significantly correlated with TPC, TEAC, FRAP and DPPH$^{\bullet}$ scavenging activity, the correlations were not as strong as with some hydroxybenzoic acids content. The highest positive associations were found between hydroxybenzoic acids content and antioxidant activity (TEAC and FRAP) values. High variation among the genotypes of the same species indicates that significant variation in terms of phenolic compounds and antioxidant activity values within *V. sativa* species can be found.

3. *V. sativa* genotypes Cvoe and Cvke differed from all other ones in terms of high TPC, antioxidant activity values, high hydroxybenzoic acids and flavones content. *V. narbonensis* all genotypes were characterised by absence of flavones and lowest TPC and antioxidant activity values.

4. Dendrograms and GGE biplots showed the differences or similarities between the genotypes according to the data of phenolic compounds and antioxidant activity assays. Absence of flavones indicated the lowest TPC, FRAP, TEAC and DPPH$^{\bullet}$ scavenging activity in the genotypes.

5. The strong correlation determined in this research shows that the TPC of *Vicia* genotypes can be helpful in determining which genotypes have higher antioxidant activity.

Acknowledgements

H. Hülya Orak thanks Council of Higher Education (YOK) in Turkey for financial support for her stay in Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences in Olsztyn.

Received 14 04 2021
Accepted 12 11 2021

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Vicia rūšių genotipų skirtumai, kuriuos lemia sėklų fenoliniai junginiai ir antioksidacinis aktyvumas

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Santrauka

Tirta įvairiose Turkijos vietovėse auginamų *Vicia* rūšių sėklų fenoliniai junginiai bei antioksidacinis aktyvumas ir analizuojamų požymių įvairovė. Tirta *Vicia* trijų rūšių: paprastojo vikio (*Vicia sativa* L.), vengrinio vikio (*Vicia pannonica* Crantz.) ir narboninio vikio (*Vicia narbonensis* L.), 9 genotipai. Eksperimentas atliktas siekiant nustatyti suminį sėklų fenolinių junginių kiekį (TPC), atskirų fenolinių junginių kiekį, $ABTS^{\bullet+}$ bei $DPPH^{\bullet}$ radikalų imobilizavimo aktyvumą ir geležies redukcijos antioksidacinę gebą (FRAP) metanolio ir acetono ekstraktuose. Vikių sėklų ekstraktų TPC buvo 11,18–30,42 mg GAE g^{-1} (metanolio ekstrakte) ir 17,05–59,88 mg GAE g^{-1} (acetono ekstrakte). Du *V. sativa* genotipai Cvoe ir Cvke išsiskyrė dideliu TPC ir antioksidaciniu aktyvumu. Juose taip pat buvo didžiausias atskirų hidroksibenzoinių rūgščių ir flavonų kiekis. Visiems *V. narbonensis* genotipų sėklų ekstraktams buvo būdinga tai, kad juose nebuvo flavonų, o TPC ir antioksidacinis aktyvumas buvo nedideli. GGE biplot analizė atskleidė *Vicia* genotipų skirtumus pagal sėklų fenolinių junginių kiekį ir antioksidacinį aktyvumą. Reikšmingos koreliacijos ($P < 0,01$) tarp TEAC, FRAP bei $DPPH^{\bullet}$ aktyvumo ir hidroksibenzoinių rūgščių kiekio nustatytos ir metanolio, ir acetono ekstraktuose. Atlikus hierarchinę klasterinę analizę genotipai buvo suskirstyti į tris acetono ekstrakto ir du metanolio ekstrakto klasterius, kurių panašumas kiekvienoje grupėje viršijo 60 %. Eksperimento rezultatai parodė, kad *Vicia* rūšių genotipų skirtumai pagal sėklų fenolinių medžiagų kiekį ir antioksidacinį aktyvumą gali būti panaudoti pašarų ruošimo technologijų, skirtų pagerinti gyvūnų mitybą bei gerovę ir mėsos kokybę, tyrimams.

Reikšminiai žodžiai: antioksidacinis aktyvumas, fenoliniai junginiai, GGE biplotas, klasterinė analizė, *Vicia* genotipai.