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Tryptophane and Raw Protein Contents of Local Pea (*Pisum sativum* L.) Lines for Different Sowing Dates

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ABSTRACT

This study was conducted to determine protein and tryptophane contents of own local peas genetic resources and identify whether there is any convenient lines to develop high nutritiousness quality variety. Fortyfour lines used in experimental study, picked before, described morphologically and selected during 6 years as making selection. These lines were sowed on two different sowing times with 4 control varieties under Samsun conditions and dried grain analyzed for their raw protein ratio and tryptophane content. Statistically significant differences were found among sowing times and tested genotypes for the raw protein ratio and tryptophane content. It was determined that both properties were higher in early spring sowing. In winter sowing, raw protein ratio ranged from 17.73 (Bz16) to 28.36% (Bz42), tryptophane content from 1936.42 (Bz1) to 3119.74 mg kg⁻¹ (Green P.). In spring sowing, raw protein ratio ranged from 19.29 (Bz2) to 27.17% (Bz 42); tryptophane content from 2231.05 (Bz31) to 2746.44 mg kg⁻¹ (Bz16). Open green grain colored, wrinkle grainy Bz42 line gave the highest value. This line was identified as the most significant candidate line for dry grain purposefully for variety development study.

Keywords: Pea; Local; Protein; Tryptophane

Farklı Zamanlarda Ekilen Yerel Bezelye (*Pisum sativum* L.) Hatlarının Triptofan ve Ham Protein İçerikleri

ESER BİLGİSİ

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ÖZET

Bu çalışmanın amacı, elimizdeki yerel bezelye gen kaynaklarının protein ve triptofan amino asiti içeriklerini belirlemek ve besleyicilik kalitesi yüksek çeşit geliştirmeye aday hatların olup olmadığını tespit etmektir. Denemede kullanılan 44 hat daha önce toplanmış, morfolojik tanımlamaları yapılmış ve 6 yıl süre ile seçilmiştir. Bu hatlar iki farklı ekim zamanında 4 kontrol çeşit ile birlikte Samsun şartlarında ekilmiş ve kuru tanelerinde ham protein oranı ve triptofan

miktarı incelenmiştir. Ham protein oranı ve triptofan miktarı üzerine ekim zamanının istatistiki olarak farklılık yarattığı tespit edilmiştir. Her iki özelliğin de erken ilkbahar ekiminde daha yüksek olduğu görülmüştür. Kışlık ekimde ham protein oranı % 17.73 (Bz16) ile % 28.36 (Bz42), triptofan miktarı 1936.42 (Bz1) ile 3119.74 mg kg⁻¹ (Green P.) arasında değişmiştir. İlkbahar ekiminde ham protein oranı % 19.29 (Bz2) ile % 27.17 (Bz 42), triptofan miktarı 2231.05 (Bz31) ile 2746.44 mg kg⁻¹ (Bz16) arasında değişmiştir. Denemede kullanılan bezelye genotiplerinin kuru tohumlarında triptofan miktarı 2167.08-2917.27 mg kg⁻¹ arasında değişmiştir. En yüksek değeri açık yeşil tohum renkli ve kırışık taneli Bz42 hattı vermiştir. Bu hat kuru tane amaçlı çeşit geliştirme çalışması için en önemli aday hat olarak tespit edilmiştir.

Anahtar Kelimeler: Bezelye; Yerel; Protein; Triptofan

1. Introduction

Proteins which compose the better part of dry substance weight of a cell and are molecules have a role in whole vital activities (Kandemir & Kavaklı 2001). Amino acids composed the protein, a human body can not synthesize the eight of them (Leucine, isoleucine, lysine, methionine, phenylalanine, threonine, tryptophane, valine). These amino acids called essential amino acids should be definitely taken on diet sufficiently. The lowest one from dietary amino acids determines protein's biological value (Akçin 1988). Each amino acids have a specific function and could prevent various illness symptoms before develop. Tryptophane balances the body's salt intake. Tryptophane is the basic material on producing painkiller neurotransmitters (serotonin, tryptamine, indoleamin and melatonine) and recognizes damaged unnatural or defective structures of DNA, and fixes them. The factor, which determines ache sense severity, is the tryptophane level that passes brain. However there is a low tryptophane in the brain, one pain such severely. Tryptophane is one of the elements giving color to the eye's iris. It works as a filter against infrared lights and severe lights that can damage the retina. Muscular movements are also significant functions of tryptophane. If there is not any tryptophane in the brain, man can not feel calm and peaceful himself (Batmanghelidj 2012). This amino acid which has so much functions should be taken with diet into the body.

The production of herbal protein isolat has started to become a significant field for industry because of raised concern to herbal proteins at a

food and out of food markets. Because of that, in particularly Europe, many countries try to develop their own protein products. The usage of protein isolats as a concentrator adding functional ingredients into food, becomes widespread (Vioque et al 1999). The most widespread usage is soya bean in this way. Including Turkey, Mediterranean basin is the origin central of many protein plants including pea. Pea flour and isolated pea protein are the examples of proteinaceous food. Isolated pea protein is a functional ingredients in terms of putting on water and fat, froth and gelling properties. It is used instead of egg whites for frozen desserts without milk and sponge cakes at the same time. Pea flour which is obtained from grinding pea grains, is a good resource because of that approximately 30% of total combination is protein (Periago et al 1998).

There is only one registered pea variety on the purpose of using as dry grain but this variety is not used at the present time. Untill today, 1 local 10 exterior registered varieties have been used on market on the purpose of fresh consumption. However, according to Akçin (1988) from Trabut (1911) and Vavilov (1950) have reported Near East and Mediterranean involved our country are also the gene centres of pea as being for many plants. It shows Turkey is lucky on the subject of developing pea varieties from local materials. As well as developable varieties are direct food, considering properties of protein concentrator addictive matter being fund food processing sector and developing.

Pea is enjoyably consumed food both in EU countries and our north and east neighbours, especially China. If Turkey have good varieties and

cultivating techniques for this product, it is possible to be exporter. Also pea is a addictive product for being founded of food processing industry. Our region has a potential that it has convenient ecology for pea cultivating and its industry can be founded and developed. Because of that, this study was conducted to determine whether there are any candidate varieties which have high protein and amino acid that is suitable for our regional conditions, and after that to breed new cultivar.

2. Material and Methods

Materials of this consists of 48 genotype as 44 line which are obtained from National Plant Gene Bank and picked from coastal city of Samsun, brought the line level with selection breeding and as the control, 4 varieties (Klein, Green Pearly, Sprinter, Sugar Bon) selected.

The study was carried out in Samsun located in Coastal Middle Black Sea Region in 2009-2010 cultivating season. Winter sowings were done in november while spring sowings in February. Some soil properties of winter sown experimental area were as follows loamy texture, notral pH (6.85), slightly saline, high organic matter while that of spring sown experimental area were as follows loamy texture, notral pH (6.89), non saline and adequate organic matter. Totally 541.2 mm rain dropped during vegetation in winter sowing period and 506.8 mm in spring sowing vegetation period.

Experiments was designed as randomized block design with three replications. Genotypes were sowed 50 x 15 cm density (each plots 5 square meters) on November 13 (winter) and February 25 (spring). Experiment was carried out in rainfed conditions without irrigation and fertilizer application. Sample was taken from blended and harvested seeds for protein and aminoacid analyses.

2.1. Raw protein ratio in seed

Protein content was determined by the Kjeldhal method using Gerhard brand instrument. Nitrogen content in the sample was converted to total protein

content by multiplying the N percentage with the standard conversion factor (6.25).

2.2. Tryptophane aminoacid quantity in seed

Tryptophane aminoacid quantity in seed was determined by alkaline hydrolysate method of Fountoulakis & Lahm (1998) whom reported it has been the most convenient method. Tryptophane stock solution (1000 mg L⁻¹) was prepared for tryptophane standard by dissolving 0.1 g of tryptophane in 100 mL of 0.1 mol L⁻¹ HCl solution. Internal standard prepared by dissolving 0.1 g of α -methyl-tryptophane in 100 mL of 0.1 mol L⁻¹ HCl solution. Seven different standard solution were prepared from stock tryptophane solution (0.001, 0.025, 0.05, 0.1, 0.25, 0.5 and 1 mL). Prepared standards being read on HPLC Thermo SP, TSP, SCM 1000. It was determined tryptophane's coming duration was at 17. min (Figure 1).

In HPLC readings, ultraviolet detector, Luna C18 (250 x 4.6 mm, size of shred 5 μ m colon was used. Colon oven temperature was adapted to 30 °C. The solution, that prepared with 3 g acetic acid, 900 mL ultra pure water, 50 mL methanol, 0.5 g 1,1,1-trichloro-2-methly-2-propanol, adjusted to pH 5 with ethanolamine solution and volume made up to 1 L with ultra pure water, was used as mobile phase. Flow rate 1 mL min⁻¹, detector's wave lenght 280 nm, injection volume 20 μ L and analyse duration as 21 min were adapted (CD 2000).

Seeds were sifted from 0.5 mm sieve as graining to determine tryptophane quantity in pea lines. 0.3000 g (containing 10 mg of nitrojen) from sifted example was weighed in capped autoclavable glass containers. 8.4 g Ba(OH)₂·8H₂O and 10 mL ultra pure water was added onto it. It was mixed in the magnetic stirrer and 4 mL ultra pure water was added, and autoclaved during 8 hours in 140 °C being closed its tubulure (Delhaye & Landry 1993). After being taken from autoclave it was shaken slightly adding 30 mL ultra pure water. In pursuit of it, 0.5 mL severe internal standard solution (α -methyl-tryptophane) was added. After that 5 mL of 0.5 mol L⁻¹ ortho-phosphoric acid solution was added. Solution was adapted to pH 3 with 1 mol

L⁻¹ HCl solution neutralizing with 6 mol L⁻¹ HCl solution. To this, after adding 25 mL methanol, its volume was completed to 100 mL with ultra pure water was transferred to 100 mL volumetric flask. Solution was read on HPLC filtered to vial from 0.45 µm filter and tryptophane quantity in seed was determined as mg kg⁻¹.

Statistical analyses were performed compounding over sowing times with respect to randomized complete block design in SPSS13 software. DUNCAN test (P<0.01) was used for multi comparisons.

3. Results and Discussion

3.1. Raw protein ratio in seed

One of most important property distinguishes legumes family from other plants made agriculture is being high protein ratio in dry seeds. Singh et al (2003) have reported the protein ratio in pea have the feature of high heredity, indicative low genetic progress and this property can be used as selection criterion. Though the height of heredity degree, environmental factors affect protein ratio. One of these factor is sowing time. Vegetation period is generally longer in winter sowing. Correspondingly, however its ratio in seed have seemed low for more dry matter processing and occurred accumulation in seed because of the high yield when calculated protein yield was found high. Accumulation of carbohydrate in seed has decreased by means of short vegetation period in early spring sowing; depending on it, protein in seed has increased proportionately, in other words quality has stood out. Biçer (1997) has reported that protein ratio in seed has changed between 23.13% and 31.31 in peas sowed at two different times as november 29 and february 16 under Diyarbakır conditions and the highest ratio was obtained from sowing on february 16. In our study as well, whereas raw protein ratio in seed in genotypes sowed in winter was averagely 20.99%, it was 22.09% for the ones sowed in early spring. This raise was found significant statistically (Table 1).

In the conclusion of performed variance analyse it was determined that raw protein ratio in seed of peas sowed in winter and early spring, affected significantly (P<0.01) from sowing dates (Table 1). Raw protein ratio in winter sowed genotypes changed between 17.73% and 28.36%. 19 genotypes were located over winter sowing experiment average. Among varieties, wrinkle grainy Sugar Bon has had the lowest raw protein ratio (22.17%) both in winter and early spring sowing and wrinkle grainy Green Pearly has had the highest ratio has given the highest value (27.17%) both at two sowing times (Table 1).

Karayel (2006) has reported the raw protein ratio is 20.4% for Sugar Bon, 19.5% for Sprinter, 19.2% for Green Pearly wintery sowed in Samsun; Bozoğlu et al (2004) have reported it has been 30.50% for Sprinter under the same conditions. Though the same ecology, being high of this difference for varieties has revealed however this property's heredity has been high, it has been affected from cultivating conditions in terms of raw protein ratio. Seed colour for pea has been one of significant quality properties determining whether it has been for cooking or not (Karayel 2012). Particularly in Turkey, light coloured seed types for legumes were preferred as for cooking. In this study, it was determined that 9 from in winter sowing lines had light colour seed, are convenient for cooking; and the others had dark colored seed were convenient for feed. Protein ratio changed between 21.82% and 27.17% in control varieties used in the experiment and Green Pearly had the highest ratio. Bz42 line which had the highest raw protein ratio, was wrinkle and light green coloured seed, passed the control varieties (Table 1).

Raw protein ratios of lines changed between 19.29% and 27.17% in early spring sowing. Bz42 line had the highest raw protein ratio and also this line passed control varieties. This value changed between 21.52% and 25.72% for varieties. As is seen, lines in comparison to varieties had a wide change in terms of protein ratio. Green Pearly had the highest protein ratio in early spring sowing as in winter sowing. The average of early spring sowing was 22.09%, 19 genotypes were passed the average

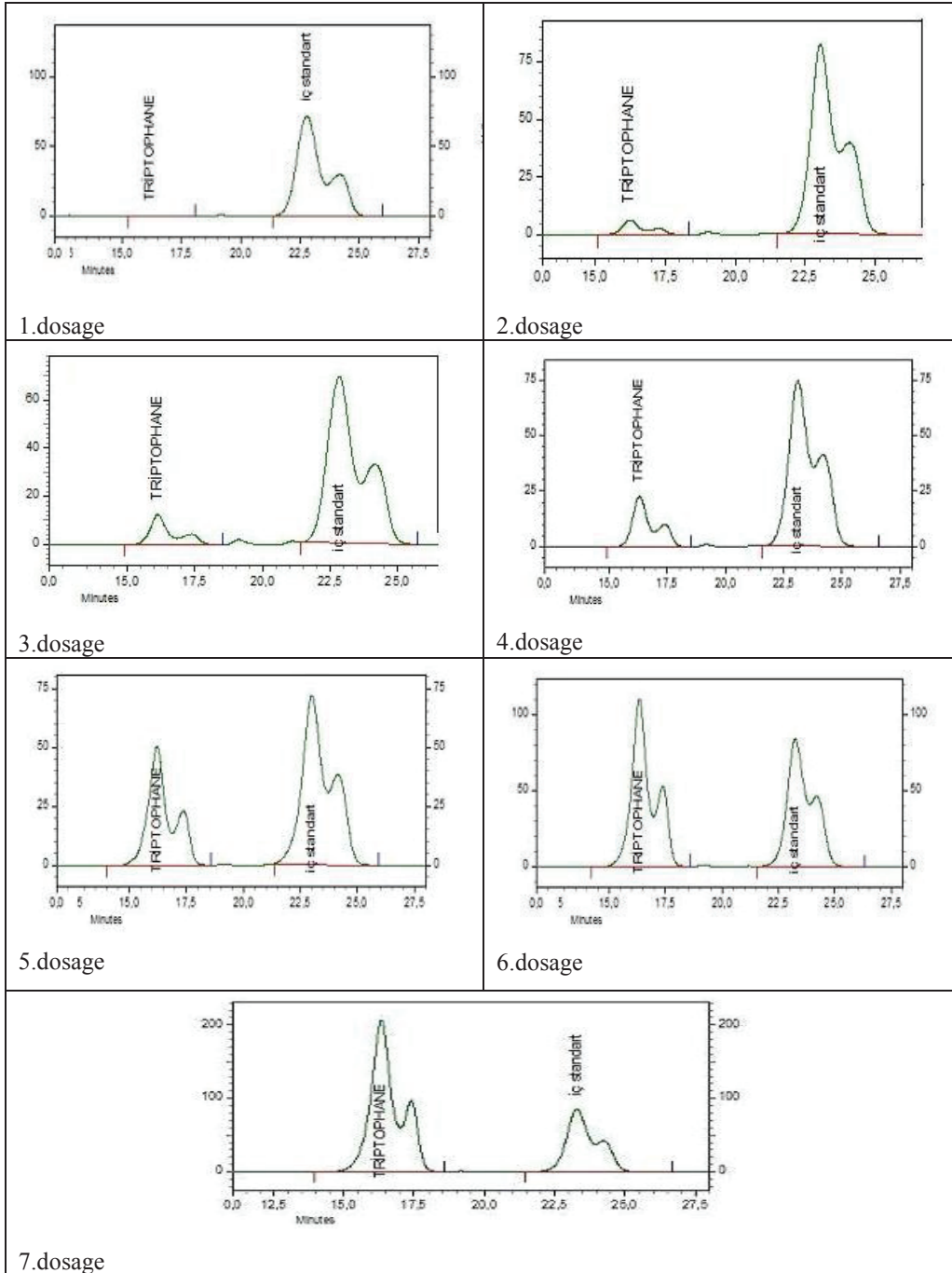


Figure 1- Chromatograms of tryptophane amino acid standarts

Şekil 1- Triptofan amino asiti standartlarının kromatogramları

Table 1- Raw protein ratios and tryptophane quantity of pea genotypes sowed in winter and early spring

Çizelge 1- Kışlık ve erken ilkbaharda ekilen bezelye genotiplerinin tanede ham protein oranları ve triptofan miktarları

Genotypes	Raw protein ratio in seed (%)			Tryptophane quantity in seed (mg kg ⁻¹)		
	Winter	Spring	Mean**	Winter	Spring	Mean**
Bz1	19.19	22.65	20.92 j-t	1936.42	2397.74	2167.08 t
Bz2	22.01	19.29	20.65 k-t	2546.35	2350.89	2448.62 e-m
Bz3	19.56	22.02	20.79 j-t	2186.61	2438.39	2312.50 l-t
Bz4	19.40	22.50	20.95 i-t	2095.75	2491.00	2293.37 m-t
Bz5	21.03	19.57	20.30 r-u	2486.89	2321.01	2403.95 f-o
Bz6	20.97	19.85	20.41 o-u	2282.81	2349.63	2316.22 k-t
Bz7	21.12	19.35	20.24 stu	2499.59	2401.76	2450.68 e-m
Bz8	19.69	21.78	20.74 j-t	2404.62	2583.82	2494.22 d-j
Bz9	18.97	19.74	19.35 u	2057.44	2346.51	2201.98 rst
Bz10	20.91	20.84	20.48 n-u	2538.52	2406.41	2472.46 d-l
Bz11	21.25	20.18	20.71 j-t	2490.65	2307.52	2399.08 f-o
Bz12	21.18	22.75	21.96 e-j	2584.98	2485.95	2535.46 c-g
Bz13	18.60	21.62	20.11 stu	2096.88	2539.39	2318.13 j-t
Bz14	22.46	19.83	21.14 h-s	2473.67	2336.27	2404.97 f-o
Bz15	21.79	22.95	22.37 d-h	2643.14	2303.56	2473.35 d-l
Bz16	17.73	25.46	21.60 f-p	2102.57	2746.44	2424.50 e-m
Bz17	21.49	23.99	22.74 def	2563.79	2664.50	2614.14 bcd
Bz18	19.80	26.57	23.18 d	2184.15	2718.06	2451.11 e-m
Bz19	19.54	21.66	20.60 k-t	2158.57	2508.81	2333.69 j-t
Bz20	21.04	22.42	21.73 f-n	2338.34	2509.93	2424.13 e-m
Bz21	20.53	22.24	21.39 g-s	2358.82	2644.43	2501.63 d-i
Bz22	20.59	22.72	21.66 f-o	2345.04	2453.03	2399.04 f-o
Bz23	21.63	21.67	21.65 f-o	2526.70	2588.10	2557.40 c-f
Bz24	18.34	21.16	19.75 tu	2221.29	2394.92	2308.10 l-t
Bz25	19.94	21.18	20.56 m-u	2308.47	2392.94	2350.71 i-r
Bz26	22.19	22.20	22.19 d-i	2705.96	2431.73	2568.85 b-e
Bz27	20.27	20.46	20.37 p-u	2332.04	2477.05	2404.55 f-o
Bz28	21.81	21.50	21.66 f-o	2489.83	2348.79	2419.31 e-n
Bz29	18.99	21.22	20.11 stu	1994.46	2381.24	2187.85 st
Bz30	19.19	22.32	20.75 j-t	2198.41	2401.89	2300.15 m-t
Bz31	19.17	20.25	19.71 tu	2189.68	2231.05	2210.37 p-t
Bz32	20.19	22.87	21.53 f-r	2293.48	2446.56	2370.02 h-p
Bz33	22.69	23.41	23.05 de	2498.28	2551.80	2525.04 c-h
Bz34	22.06	20.58	21.32 g-s	2507.85	2280.58	2394.21 f-o
Bz35	20.33	21.29	20.81 j-t	2421.59	2536.76	2479.17 d-k
Bz36	21.62	21.84	21.73 f-n	2132.26	2385.27	2258.76 n-t
Bz37	21.91	20.46	21.19 h-s	2390.19	2294.11	2342.15 i-s
Bz38	18.31	22.83	20.57 l-u	2144.69	2593.89	2369.29 h-p
Bz39	21.24	19.48	20.36 p-u	2502.59	2359.76	2431.18 e-m
Bz40	20.47	20.78	20.63 k-t	2412.69	2383.62	2398.15 f-o
Bz41	20.12	24.95	22.54 d-g	2156.34	2585.87	2371.10 g-p
Bz42	28.36	27.17	27.77 a	2711.88	2710.45	2711.16 b
Bz43	25.46	24.51	24.98 c	2674.26	2657.67	2665.97 bc
Bz44	18.94	24.77	21.85 f-l	2172.61	2518.30	2345.46 i-s
Klein	21.82	21.92	21.87 f-k	2074.25	2416.93	2245.59 o-t
Sugar B.	22.17	21.52	21.84 f-m	2374.20	2596.52	2485.36 d-j
Sprinter	24.31	25.16	24.73 c	2545.95	2716.51	2631.23 bcd
Green P.	27.17	25.72	26.44 b	3119.74	2714.81	2917.27 a
Mean**	20.99 B	22.09 A		2364.07 B	2472.96 A	

**, P < 0.01

and 10 of them had dark color seed. Lines had the lowest protein ratio both in two sowing, had small, dark green colored seed, black hilum, short pod were feed type, were not convenient for cooking. These lines are suggested as feed considering rich protein content. Timuroğlu et al (2004) have determined raw protein ratio of some pea lines which were sowed as summery and developed as feeder under Ankara conditions changed between 16 and 23.5%. Seyis (1994) has determined raw protein ratio of summer sowed pea varieties in Samsun changed between 18.72 and 24.37% and also Gülümser et al (1994) have determined it changed between 19.75 and 24.01% at the same ecology.

In the conclusion of variance analyse performed for the raw protein ratio in seed of 48 genotype (for line and control) sowed in winter and early spring, the difference ($P<0.01$) was seen between genotypes and in terms of sowing time x genotype interaction. When lines were evaluated without sowing times, raw protein ratio in seed changed between 19.35%

and 27.77% and for varieties 21.84%-26.44% and it is monitored local materials passed control varieties. Bz9 lines had the lowest raw protein ratio in seed, had the feature of brownish green, dimpled whereas the highest line is Bz42 which had the highest raw protein ratio, had the feature of light green, wrinkle seed both in winter and early spring sowing. This type looked appropriate for cooking pea cultivation.

3.2. Tryptophane aminoacid quantity in seed

In the conclusion of variance analyses it was determined there was statistically a difference ($P<0.01$) between winter and early spring sowing in terms of tryptophane quantity of pea genotypes. Tryptophane quantity in seed was found more high in early spring sowing than in winter sowing (Table 1). Being high of protein ratio in early spring sowing as well resulted in that conclusion.

Showing chromatograms tryptophane values of some genotypes in winter sowing were given at Figure 2. Tryptophane quantity of Bz26, Bz44

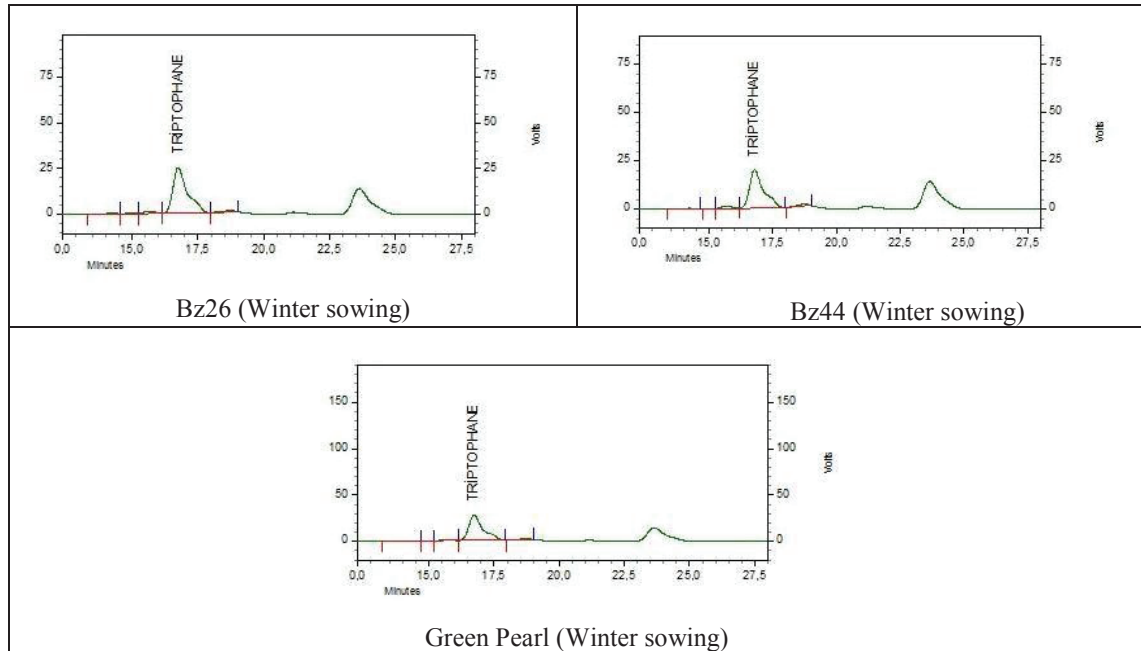


Figure 2- Tryptophane chromatograms of some genotypes in winter sowing

Şekil 2- Bazı genotiplerin kışlık ekimdeki triptofan kromatogramları

and Green Pearly, which cromotography can be seen in Figure 2, were determined 2705.96, 2172.61 and 3119.74 mg kg⁻¹, respectively. Tryptophane quantity of lines in winter sowing changed between 1936.42 and 2711.88 mg kg⁻¹ (1.009-0.956 g 16 g⁻¹ N), whereas 2074.25-3119.74 mg kg⁻¹ (0.951-1.148 g 16 g⁻¹ N) for control varieties. In early spring sowing tryptophane quantity of lines changed between 2231.05 and 2746.44 mg kg⁻¹ (1.102-1.079 g 16 g⁻¹ N), whereas 2416.93-2716.51 mg kg⁻¹ (1.103-1.080 g 16 g⁻¹ N) for control varieties. The highest quantity of tryptophane (3119.74 mg kg⁻¹-1.148 g 16 g⁻¹ N) was encountered in winter sowing Green Pearly. Some genotypes' tryptophane quantity belonging to HPLC chromatograms were given at Figure 2. Chavan et al (1999) determined tryptophane quantitative in *Pisum sativum* as 0.2 ± 0.01 g 16 g⁻¹ N; Wang & Daun (2004) determined tryptophane quantitative in Canada pea as 07-09 g 16 g⁻¹ N; Yemane & Skjelvag (2003) determined it as 1.14 g 16 g⁻¹ N in Dekoko pea (*Pisum sativum* var. *abyssinicum*); as 1.10 g 16 g⁻¹ N in Ater (*Pisum sativum* var. *sativum*). Khattab et al (2009) determined there was 0.61-0.86 g tryptophane in 100 g protein of uncured dry pea in their study researching feed quality of legume seeds some physical applicants done. Iqbal et al (2006) investigated the feed quality of significant edible legumes (chickpea, lentil, cowpea, green pea). They have determined tryptophane quantitative in green pea's protein as 0.8 ± 0.02%.

In the conclusion of variance analyses performed for tryptophane quantity in seed of 48 genotypes sowed in winter and early spring, there was a different P<0.01 level between genotypes and sowing times x genotype interaction. The highest tryptophane quantity was in Bz42 line that was light green and wrinkle, its tryptophane quantity was 2711.16 mg kg⁻¹ (0.976 g 16 g⁻¹ N). Bz17, Bz26, Bz43 lines and also Sprinter control variety were placed in the same statistical group. Bz42 line except Green Pearly (2917.27 ppm) had higher tryptophane quantity than other control varieties.

4. Conclusions

The raw protein ratio, one of the most important properties of legumes changed 19.35-27.77% for genotypes and it is monitored local materials passed control varieties. Bz42 line is the most remarkable material due to higher protein ratio (27.77%) than Green Pearly control variety, which the highest protein ratio (26.44%) has become. Besides, tryptophane quantitative of lines have changed 2167.08-2711.16 mg kg⁻¹. Having the highest tryptophane quantitative of Bz42 has had the feature of light green colored, wrinkle seed, can be suggested to develop variety convenient on the purpose of dry grain.

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