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Identification of Morphological Agronomic and Quality Characteristics of Hungarian Vetch (*Vicia pannonica* Crantz.) Mutants

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ABSTRACT

Gamma-ray irradiation of seeds is an effective method for creating genetic variation in plant breeding. This study aimed to evaluate the morphological, agronomic, and quality characteristics of Hungarian vetch (*Vicia pannonica* Crantz.) mutant lines obtained through gamma radiation. The experiment was carried out in Golbaşı-Ankara during the 2019 and 2020 growing seasons according to Randomized Complete Block Design with four replications involving eleven mutant lines and two control cultivars. The mutant lines exhibited a range of outcomes, including both positive and negative effects. However, no significant alterations were observed in the quality characteristics. The TB1007 mutant line demonstrated superior yield and quality, whereas the TB1002 and TB1005 mutant lines only performed well in terms of yield. The highest hay yields were obtained from TB1007 and TB1002, with 42.50 and 41.93 kg ha^{-1}, respectively, followed by the cv. Tarm Beyazi-98 and TB1005 mutant, with 39.14 and 39.01 kg ha^{-1}, respectively, in 2019. The TB1007 mutant line had the highest digestible protein content, with a content of 16.4% in 2019 and 16.7% in 2020, averaging at 16.5%. These lines are suitable for breeding programs that, especially studies involving crosses.

1. INTRODUCTION

The *Vicia* genus, which belongs to the Fabaceae family, includes nearly 200 species, many of which are winter annual legumes commonly referred to as vetches (Renzi et al., 2020; Acikgoz, 2021). Vetches are valuable the forage crops of economic importance (Zhang and Mosjidis,1998) and are also known for improving soil fertility (Acikgoz, 2021). These crops in dry regions with continental climates, where their high production potential makes them an excellent choice for forage (Uzun et al., 2004, Sahin-Demirbag et al., 2008, Erkovan, 2022).

Hungarian vetch (*Vicia pannonica* Crantz.) is commonly cultivated as a winter crop due to its high forage yield potential and resistance to both cold and drought. Its cultivation has been expanding in semi-arid regions, particularly in Türkiye. A diploid annual legume (2n=2x=12) Hungarian vetch is found across Southern and Central Europe, Anatolia, North-West Africa, and Caucasia (Naydenova and Aleksieva, 2014; Acikgoz, 2021). This species adapts well to the continental climate, and is planted in autumn, even in areas with harsh winters, such as Central and Eastern Anatolia (Acikgoz, 2021). Hungarian vetch is mainly grown for hay production, though its seeds are also valuable in poultry feed. In Türkiye’s semi-arid regions, forage yields of Hungarian vetch range from 7.5 to 35 tons per hectare, while hay yields range from 2 to 7 tons per hectare (Unal et al., 2011; Erdogdu et al., 2016). Yield varies greatly depending on environmental conditions and genotypes. The wide genetic diversity of genotypes is a key element for an effective and efficient breeding programme focusing on yield-related traits (Ergun et al., 2023). However, many forage crops, including vetches, suffer from limited genetic diversity, restricting breeding efforts (De la Rosa et al., Acikgoz, 2021).

Mutation breeding is a key method in plant breeding for enhancing genetic resources (Chaudhary et al. 2019; Shi et al., 2020; Sarsu et al. 2021). Mutations, sudden changes in a plant's genetic structure, can be induced using physical or chemical mutagens (Karim et al., 2008; Acikgoz, 2021). Plant breeding programs utilize both direct and indirect mutation methods to generate new traits, which can then be used for further crossbreeding and genetic improvement (Sagel et al., 1994; Sehirali and Ozgen, 2007; Sabancı and Ayrancı, 2015; Cimen et al., 2019). However, only five mutant cultivars have been released in fodder crops to date, which is relatively few (Acikgoz, 2021). Notable examples of vetch cultivars developed through mutation breeding include the common vetch cultivars Nechinovskaya 84 and Nikian, which were made using direct mutation breeding, and the Toplesa variety, developed through the use of a mutant line in crossing. Additionally, two vetch lines, VSMP 04 and Mutant Line No. 1, have shown improvements in grain yield. (Micke, 1993; Artık and Pekşen, 2006; Aleksieva and Naidevova, 2012; Efe and Unal, 2017; Mikić et al., 2019).

Mutation breeding of Hungarian vetch began in 2010 at the Field Crops Central Research Institute, to increase genetic variation. The test material, Tarm Beyazi-98, was exposed to various doses of Gamma radiation. Plants with desirable traits were selected from the M_2 to M_4 generations, and by the M_5 generation, line selection and seed multiplication had begun. In 2019 and 2020, yield trials were conducted with 11 mutant lines and two control cultivars.

The primary objectives of this research were to (1) investigate the morphological, agronomical, and forage quality traits of the mutant lines under semi-arid conditions, (2) compare the mutant lines to control cultivars in order to assess the effects of the mutations, and (3) provide detailed data on the development of genetic diversity, particularly focusing on yield and quality traits.

2. MATERIAL AND METHOD

Material

Eleven mutant lines and two control cultivars (cv. Kansur and cv. Tarm Beyazi-98) were used as plant material in the study (Table 1).

Table 1. The mutant lines and control cultivars of Hungarian vetch

Number	Lines Cultivars	Doses (Gy)	Mutant lines
1	Tarm Beyazi-98	60	TB603
2	Tarm Beyazi-98	60	TB605
3	Tarm Beyazi-98	80	TB802
4	Tarm Beyazi-98	80	TB805
5	Tarm Beyazi-98	80	TB806
6	Tarm Beyazi-98	100	TB1002
7	Tarm Beyazi-98	100	TB1005
8	Tarm Beyazi-98	100	TB1006
9	Tarm Beyazi-98	100	TB1007
10	Tarm Beyazi-98	100	TB1008
11	Tarm Beyazi-98	100	TB1009
12	Kansur	-	Control
13	Tarm Beyazi-98	-	Control

The soil condition at experimental area was slightly alkaline and poor in organic matter while the levels of P2O5, K2O, and CaCO3 were relatively high.

Rainfall in spring and autumn is necessary for germination, emergence, growth, and development of plants in Hungarian vetch cultivation in the Central Anatolian region. Rainfall in the experimental years was lower than the long-term average (Table 2) (Anonymous, 2021). 2020 also received more precipitation than the first year, especially higher amounts in April, May and June. Temperature values were lower than the long-term average in the first year and higher in the second year. Relative humidity was below the long-term average in both years.

Table 2. Climatic data of experimental field (TSMS, 2021)

Mounths	Precipitation (mm)			Temperature (ºC)			Relative moisture (%)		
	2019	2020	LT*	2019	2020	LT	2019	2020	LT
January	36.2	17.4	32.5	-0.8	-2.2	-1.4	83.9	74.8	88.2
February	36.4	33.2	22.7	2.2	0.5	1.0	75.0	70.9	80.9
March	20.6	14.2	40.3	5.0	5.6	5.1	59.2	60.7	71.2
April	23.4	39.0	24.5	7.9	8.2	9.6	61.1	54.8	63.6
May	3.8	40.6	47.5	15.1	13.3	14.3	55.4	55.9	62.6
June	15.0	74.3	38.1	18.7	17.3	18.5	58.7	53.7	58.9
July	7.2	0.6	9.5	17.8	23.1	22.2	54.3	42.3	46.1
August	9.2	0.0	8.1	20.8	22.7	22.5	47.1	37.5	45.0
September	4.2	11.6	18.5	16.3	20.9	18.0	47.2	47.8	49.5
October	9.2	20.0	33.0	12.0	15.6	12.0	54.6	54.3	64.6
November	35.2	2.4	15.6	6.7	4.5	5.6	64.4	77.8	74.9
December	21.4	10.2	28.1	0.9	4.0	0.8	83.6	86.7	85.8
Total/Average	221.80	263.50	318.40	10.22	11.13	10.68	62.04	59.77	65.94

Method

Hungarian vetch cultivar, Tarm Beyazi-98, was performed for Cobalt-60 (Co60) gamma irradiation at different doses (40, 60, 80, 100, 120, and 140 Gy) (105 seeds for each dose) in 2010 at the Turkiye Atomic Energy Authority (TAEK), Ankara Nuclear Research and Training Centre (ANAEM). M1 plants were grown in bulk. In the following generations (M2, M3, M4), observations were made on the mutant plants and the plants with the desired characteristics were carried to the next generation. When M5 generation was achieved, line selection and seed multiplication were started. However, as lines with the desired performance could not be obtained with 40, 120 and 140 gamma ray doses, these lines are not included in the present study. During the 2016-2018 growing seasons, sufficient seed of the mutant lines was produced to conduct the field yield trial. At this stage, the performance of the mutant lines was determined to be used in the further breeding process. In 2019 and 2020 growing seasons, yield trials were conducted in the research field of the Field Crops Central Research Institute, Ankara.

This research was carried out in a Randomized Comlepte Block Design with four replications in the field of Field Crops Central Research Institute Gölbaşı-İkizce Agricultural Research and Application Field in dry conditions in 2019 and 2020 growing seasons. The seeds of the mutant lines and the check cultivars were sown by hand in plots consisting of 6 rows with a row spacing of 25 cm. The length and width of the plots were fixed at 5 m and 1.5 m respectively. A distance of 2 m between blocks and 0.5 m between plots was left. At the same time as planting, DAP fertiliser was applied at a rate of 100 kg per hectare.

Morphological and agronomic data were collected between 15 May - 21 June 2019 and 21 May - 2 July 2020. The following morphological and agronomical traits were determined: natural plant height habitus (NPH) (cm), plant height (PH) (cm), stem diameter (SD) (mm), stem number (SN), pods per plant (PPP) (number), seeds per pod (SPP) (number) and 1000 seed weight (SW) (g), forage yield (FY) (kg ha^{-1}), hay yield (HY) (kg ha^{-1}), biological yield (BY) (kg ha^{-1}), grain yield (GY) (kg ha^{-1}) and harvest index (HI) (%). The quality traits of dry matter (DM) (%), digestible dry matter (DDM) (%), crude protein (CP) (%), digestible crude protein (DCP) (%), Acid Detergent Fibre (ADF) (%), Neutral Detergent Fibre (NDF) (%) and Relative Feed Values (RFV) (%) were identified through NIRS (Forage calibration package Lhay5fe.eqa). The significance levels of the differences between the examined traits of the mutant lines and check cultivars were determined using two-year combined analysis of variance (ANOVA) and then the genotypes were grouped according to Student's t multiple comparison tests (LSD) at 5% probability. Hierarchical cluster analysis (HCA) was performed to determine the similarities between the mutant lines and the control cultivars. Correlation-based Principal Component Analysis (PCA) was also used to examine the relationships between characters and to identify the dominant genotypes with respect to these characters. HCA and PCA were carried out using Jump 11 statistical software.

3. RESULTS AND DISCUSSIONS

Morphological traits

The natural plant height habitus, plant height, stem diameter, stem number, pods per plant, number of seeds per pod and 1000 seed weight (ANOVA) results of mutant lines and check cultivars are given in Table 3. Combined ANOVA results showed that there weren't significant differences among study genotypes for morphological traits in Table 3. Year effects seemed significant for stem number (P<0.01) and 1000 seed weight (P<0.01). No significant differences appeared on year x genotype interactions for traits.

Table 3. The combined variance analysis results (mean squares) of natural plant height habitus, plant height, stem diameter, stem number, pods per plant, number of seeds per pod and 1000 seed weight

Source	DF	Natural plant height habitus (cm)	Plant height (cm)	Stem diameter (mm)	Stem number	Pods per plant (number)	Seeds per pod (number)	1000 seed weight (g)
Years	1	38.02	206.13	0.08	43.16**	61.85	6.01	1599.96**
Error 1	3	65.41	48.15	0.30	0.32	26.28	0.73	2.54
Genotypes	12	30.35	41.52	0.06	0.21	24.42	0.37	18.02
Years x Genotypes	12	29.13	39.82	0.05	0.30	18.67	0.51	13.69
Combined error	72	31.37	36.25	0.05	0.25	13.25	0.51	18.68

** Significant at 1%; DF Degrees of freedom

Table 4. Natural plant height habitus, plant height, stem diameter and stem number of the standard varieties and mutant lines in 2019, 2020 and two-years averages

Genotypes	Natural plant height habitus (cm)			Plant height (cm)			Stem diameter (mm)			Stem number		
	2019	2020	Ave.	2019	2020	Ave.	2019	2020	Ave.	2019	2020	Ave.
TB603	36.3	37.9 bc	37.1	41.8	42.1 b	42.0	2.3	2.4	2.3	3.7	3.0	3.3
TB605	38.7	37.2 bc	38.0	43.2	41.9 b	42.6	2.2	2.2	2.2	3.8	2.6	3.2
TB802	38.4	36.0 bc	37.7	44.1	40.8 b	42.4	2.1	2.3	2.2	3.7	2.4	3.0
TB805	41.7	36.9 bc	39.3	47.7	39.8 bc	43.8	2.7	2.3	2.5	3.8	2.5	3.1
TB806	36.3	36.9 bc	36.6	41.7	40.2 bc	40.9	2.3	2.4	2.3	4.0	2.6	3.3
TB1002	39.8	36.2 bc	38.0	45.5	40.7 b	43.1	2.2	2.2	2.2	3.9	2.2	3.0
TB1005	40.6	39.8 b	40.2	45.9	42.9 b	44.4	2.2	2.3	2.3	3.8	2.6	3.2
TB1006	39.1	32.1 c	35.6	44.4	33.2 c	38.8	2.1	2.3	2.2	3.4	2.3	2.8
TB1007	41.2	36.9 bc	39.1	48.9	42.7 b	45.8	2.2	2.3	2.2	4.4	2.2	3.3
TB1008	38.9	47.0 a	42.9	44.4	51.4 a	47.9	2.4	2.4	2.4	4.1	2.8	3.4
TB1009	38.5	37.8 bc	38.2	43.3	44.3 ab	43.8	2.1	2.3	2.2	3.3	2.7	3.0
Kansur	34.9	36.6 bc	35.7	41.8	40.2 bc	41.0	2.2	2.3	2.3	3.8	2.5	3.1
Tarm Beyazi-98	39.5	35.9 bc	37.7	44.6	40.5 b	42.6	2.3	2.3	2.3	3.9	2.5	3.2
Mean	38.8	37.6	38.2	44.4	41.6	43.0	2.2	2.3	2.3	3.8	2.5	3.1
LSD. $_{(0.05)}$	9.3	6.5	5.6	10.0	7.1	6.0	0.4	0.2	0.2	0.8	0.7	0.5
CV. (%)	16.8	12.0	14.7	15.6	11.9	14.0	13.0	6.8	10.3	14.3	18.4	15.9

LSD Least significant differences; CV (%) Coefficient of variation

When the natural plant height habitus (NPH) is examined, the highest and lowest values were obtained from the genotypes TB1008 (42.9 cm) and TB1006 (35.6 cm), respectively (Table 4). NPH in Hungarian vetch was reported to change between 35.7-54.0 cm (Aggunlu, 1999). The data in present study are in agreement with the researcher. The highest plant height (PH) was obtained from line TB1008 (47.9 cm), followed by mutant line TB1007 (45.8 cm). The lowest PH was measured by the line numbered TB806 (40.9 cm) (Table 4). PH results are also similar to the findings of Sayar et al. (2012); Eviz (2016); Hashalıcı et al. (2017) and Tankus (2020). It was observed that the mutation had no effect on stem diameter (SD). SD varied between 2.2 and 2.5 mm (Table 4). The data of the present study are consistent with the findings of different researchers such as Sayar (2014); Efe and Unal (2017). It has been determined that the mutagen does not significantly affect stem number (SN), but it does affect plant height (PH). The stem count ranged from 2.8 to 3.4 (Table 4). These findings are consistent with previous studies conducted by Sayar (2014); Efe and Unal (2017).

Table 5. Number of pods per plant, number of seeds per pod and 1000 seed weight of the standard of varieties and mutant lines in 2019, 2020 and two-year averages

Genotypes	Pods per plant (number)			Seeds per pod (number)			1000 seed weight (g)		
	2019	2020	Ave.	2019	2020	Ave.	2019	2020	Ave.
TB603	13.6	13.1	13.4	4.7	5.1	4.9	46.0	55.0	50.5
TB605	11.5	15.7	13.6	4.4	5.2	4.8	46.8	53.0	49.9
TB802	12.8	15.8	14.3	4.9	5.0	4.9	51.6	54.7	53.1
TB805	13.2	20.0	16.6	4.9	5.2	5.0	48.5	55.9	52.2
TB806	14.8	15.6	15.2	4.5	5.3	4.9	48.9	53.5	51.2
TB1002	14.3	16.9	15.6	4.3	5.1	4.7	49.6	53.9	51.7
TB1005	17.9	15.4	16.6	4.8	4.5	4.6	45.8	55.3	50.6
TB1006	15.2	14.2	14.7	4.9	4.7	4.8	44.2	54.2	49.2
TB1007	15.5	18.0	16.7	4.8	4.6	4.7	44.9	55.5	50.2
TB1008	12.2	17.1	14.6	4.5	5.2	4.8	43.9	54.6	49.3
TB1009	12.9	14.4	13.7	4.4	5.3	4.8	43.8	54.9	49.3
Kansur	13.4	9.1	11.3	4.6	6.1	5.4	44.3	52.3	48.3
Tarm Beyazi-98	10.7	12.8	11.7	4.2	4.7	4.5	44.3	51.8	48.0
Mean	13.7	15.2	14.4	4.6	5.1	4.8	46.4	54.2	50.3
LSD. (0.05)	5.0	5.4	3.6	1.0	1.1	0.7	6.7	5.6	4.3
CV. (%)	25.5	24.9	25.2	15.1	14.6	14.8	10.1	7.2	8.6

LSD Least significant differences; CV (%) Coefficient of variation

The mutagen effect was higher in PPP than in other traits and higher variation occurred for this trait. Therefore, coefficient of variation was found to be high in PPP. According to the average results, the lowest, highest and average PPP were 11.3, 16.7, and 14.4 respectively. The number of SPP ranged from 4.5 to 5.4, with an average of 4.8. SW changed from 48.0 to 53.1 g and the average value had 50.3 g. When Tarm Beyazi-98 had 11.7 PPP, mutant lines were found between 13.4 and 16.7. The same situation was observed in SW. While SW in Tarm Beyazi-98 was 48 g, this value reached up to 53.1 g in mutant lines. The increase in these two traits in mutant lines naturally led to increased seed yield. When PPP was evaluated TB1007 (16.7 number) gave the highest number of pods. This was followed by line TB1005 (16.6 number). The lowest PPP was obtained from the control cultivar Kansur (11.3 number) (Table 5). In a study of Diyarbakır conditions, PPP was determined to vary between 15.6 and 29.5 (Seydosoglu, 2014). PPP values we obtained are in agreement with the researcher. SPP and SW values were 4.5-5.4 number and 48.0-53.1 g respectively. In the previous study SPP was counted as 2.8-5.7 number (Efe and Unal, 2017) and 2.5-3.2 number (Seydosoglu, 2014). SW values were also detected to change from 29.9 to 46.6 g in the past study (Seydosoglu, 2014).

It is widely acknowledged in the scientific literature that the effects of mutation applications vary considerably according to the species, variety, and line of the plant in question. Furthermore, these effects can be either positive or negative in terms of the plant's physiological, morphological, and agricultural characteristics (Aleksieva ve Naidevova, 2012; Efe ve Unal, 2017; Ogutcen et al., 2018). The results of this study indicate that the impact of gamma-ray treatment on morphological and agricultural characteristics in mutant lines is variable. This finding is consistent with other studies in this area.

Agronomic traits

The results of the variance analysis for forage and hay yields, biological yield, grain yield and harvest index in the genotypes are presented in Table 6. As indicated in Table 6, significant differences were identified between the genotypes for biological yield and harvest index at the $p < 0.01$ level. A significant difference ($P < 0.01/P < 0.05$) was observed for all traits in the years x interactions category. There was no significant difference between years at observed traits in Table 6. However, year values are given to show similarities. Evaluations and comments were made based on general average values. While there was no difference in forage yields between genotypes in 2020, there were significant differences in 2019 data, so they were grouped. Furthermore, genotypes responded differently to years, so the year x genotype interaction was significant.

Table 6. The combined variance analysis results (mean squares) of forage and hay yields, biological yield, grain yield and harvest index

Source	DF	Forage yield (kg ha^{-1})	Hay yield (kg ha^{-1})	Biological yield (kg ha^{-1})	Grain yield (kg ha^{-1})	Harvest index (%)
Years	1	2970.01	2294.11	24.94	5.41	15.70
Error 1	3	3142.24	250.51	105.64	7.51	22.68
Genotypes	12	939.48	78.02	67.32**	2.67	70.05**
Years x Genotypes	12	1165.30*	85.60*	86.77**	4.37**	115.40**
Combined error	72	519.42	43.61	25.70	1.52	19.15

* Significant at 5%; ** Significant at 1%; DF: Degrees of freedom

Table 7. Forage and hay yields of the standard varieties and mutant lines in 2019, 2020 and two-year averages

Genotypes	Forage yield (kg ha^{-1})			Hay yield (kg ha^{-1})		
	2019	2020	Ave.	2019	2020	Ave.
TB603	100.87 bcd	98.29	99.58	32.48 abc	24.57	28.53
TB605	101.87 bcd	79.58	90.73	31.46 bc	19.90	25.68
TB802	90.71 cd	98.63	94.67	27.12 c	24.66	25.89
TB805	116.67 abcd	76.08	96.38	34.15 abc	19.02	26.59
TB806	91.47cd	90.00	90.73	30.84 bc	22.50	26.67
TB1002	131.13 ab	95.61	113.37	41.93 a	23.90	32.92
TB1005	129.53ab	105.39	117.46	39.01 ab	26.35	32.68
TB1006	111.60 abcd	79.72	95.66	34.74 abc	19.93	27.33
TB1007	140.60 a	95.83	118.22	42.50 a	23.96	33.23
TB1008	100.13 bcd	125.96	113.05	31.54 bc	31.49	31.51
TB1009	87.33 d	110.39	98.86	27.12 c	27.60	27.36
Kansur	92.53 cd	105.50	99.02	29.36 bc	26.38	27.87
Tarm Beyazi-98	121.60 abc	116.11	118.86	39.14 ab	29.03	34.08
Mean	108.93	98.24	103.58	33.95	24.56	29.26
LSD. $_{(0.05)}$	31.20	34.11	22.72	10.33	8.53	6.58
CV. (%)	19.97	24.21	22.00	21.2	24.21	22.57

LSD Least significant differences; CV (%) Coefficient of variation

According to the results of the combined analysis of the Tarm Beyazi-98 had the highest forage yields (FY) (118.86 kg ha^{-1}) and the TB605 and TB806 was the lowest FY (90.73 kg ha^{-1}) (Table 7). FYs were reported as 122.7-233.6 kg ha^{-1} in Sayar et al. (2012), 100.39-173.39 kg ha^{-1} in Tankus (2020), and 116.07-260.0 kg ha^{-1} in Hashalıcı et al. (2017). As the present study results were smaller than the data of Sayar et al. (2012), but they were similar to the values of Tankus (2020) and Hashalıcı et al. (2017). The combined analysis of Tarm Beyazi-98 produced the highest hay yields (HY) (34.08 kg ha^{-1}) while TB605 had the lowest HY (25.68 kg ha^{-1}) (Table 7). These research results correspond with the findings of Sayar et al. (2012), Eviz (2016), Hashalıcı et al. (2017), Tankus (2020), Ertekin et al. (2020).

Table 8. Biological yield. grain yield and harvest index of the standard varieties and mutant lines in 2019, 2020 and two-year averages

Genotype	Biological yield (kg ha^{-1})			Grain yield (kg ha^{-1})			Harvest index (%)		
	2019	2020	Ave.	2019	2020	Ave.	2019	2020	Ave.
1 (TB603)	24.03 abcd	20.42 de	22.23 c	3.72 de	5.20	4.46	16.1 cd	26.0 a	21.0 b
2 (TB605)	24.22abcd	26.13 cde	25.18 abc	4.59 abcde	4.68	4.63	18.9 cd	17.7 cd	18.3 bc
3 (TB802)	22.77 cd	20.96 cde	21.86 c	4.17 cde	4.31	4.24	18.9 cd	20.4 bc	19.6 b
4 (TB805)	22.37 cd	27.17 abcd	24.77 abc	3.81 de	4.72	4.26	17.4 cd	17.1 cd	17.3 bc
5 (TB806)	20.87 d	22.95 cde	21.91 c	5.59 abc	3.00	4.29	27.2 ab	13.6 d	20.5 b
6 (TB1002)	18.47 d	24.75 cde	21.61 c	6.29 a	5.35	5.82	34.0 a	21.1 abc	27.6 a
7 (TB1005)	29.13 abc	18.77 e	23.95 bc	5.07 abcd	4.87	4.97	16.8 cd	25.4 a	21.1 b
8 (TB1006)	28.89 abc	28.20 abc	28.54 ab	6.19 ab	4.95	5.57	21.6 bc	18.0 cd	19.8 b
9 (TB1007)	30.60 a	26.69 bcd	28.64 ab	5.04 abcd	5.30	5.17	17.0 cd	19.8 bc	18.4 bc
10 (TB1008)	29.91 ab	21.27 cde	25.59 abc	3.83 de	5.20	4.51	13.1 d	24.5 ab	18.8 bc
11 (TB1009)	24.84 abcd	34.73 a	29.79 a	4.57 bcde	6.73	5.65	18.8 cd	19.4 c	19.1 bc
12 (Kansur)	23.27 bcd	33.82 ab	28.54 ab	3.17 e	5.51	4.34	13.4 d	16.4 cd	14.9 c
13 (Tarm Beyazi-98)	21.87 d	28.12 abc	24.99 abc	3.30 e	5.43	4.36	15.4 cd	19.2 c	17.3 bc
Mean	24.71	25.69	25.20	4.56	5.02	4.79	19.1	19.9	19.5
LSD. $_{(0.05)}$	6.91	7.61	5.05	1.71	1.83	1.23	7.37	4.95	4.36
CV. (%)	19.50	20.66	20.12	26.10	25.42	25.76	26.87	17.33	22.43

LSD Least significant differences; CV (%) Coefficient of variation

According to the average biological yields (BY) the TB1002 and TB1009 had the lowest and highest averages at 21.61 and 29.79 kg ha^{-1} respectively (Table 8). In a previous study it was found as 19.69 – 39.07 kg ha^{-1} (Cebeci, 2017). Furthermore some study results were reported that BY in different Hungarian vetch varieties were found to be 18.8-26.4 kg ha^{-1} (Ucar, 2022) and 27.20 kg ha^{-1} (Koç, 2020). The findings of these studies align with each other. Based on the two-year averages the TB802 and TB1002 had the lowest and highest grain yields (GY) at 4.24 and 5.82 kg ha^{-1} respectively (Table 8). Similar to this study Koç (2020) calculated GY of Hungarian vetch to be 5.32 kg ha^{-1}. A comparison of the cultivar (Tarm Beyazi-98) with GY of the lines (TB1002, TB1009, TB1006, TB1007 and TB1005) revealed that the lines exhibited higher yields, ranging from 13.99 to 33.49%. It is thought that the high GY can be explained by mutations impacting PPP and SW (Sattler et al., 2014; Shi et al., 2020).This increase in GY clearly shows the positive effect of mutation application (Fang et al., 2012). The averages changed from 14.9% (Kansur) to 27.6% (TB1002) in harvest index (HI) (Table 8). In a study comparable to this one Koç (2020) determined that HI for Hungarian vetch was 19.58%.

No significant differences were observed between the years for the five aforementioned traits. The genotypes exhibited disparate responses to varying rainfall and temperature levels in both years, resulting in notable alterations in their yearly rankings. Consequently, the genotype-by-year interaction was found to be statistically significant at the 1 or 5% level.

Quality traits

Variance analysis results of of dry matter, digestible dry matter, crude protein, digestible protein, acid detergent fiber, neutral detergent fiber and relative feed values for genotypes are given in Table 9. When Table 9 was observed there was no significant difference between the genotypes, years and year x genotype interaction in terms of these four traits.

Table 9. The combined variance analysis results (mean squares) of dry matter, digestible dry matter, crude protein, digestible protein, acid detergent fiber, neutral detergent fiber and relative feed values

Source	DF	Dry Matter (%)	Digestible Dry Matter (%)	Crude Protein (%)	Digestible Protein (%)	Acid Detergent Fiber (%)	Neutral Detergent Fiber (%)	Relative Feed Values
Years	1	0.17	2.10	0.83	0.35	3.46	32.05	288.21
Error 1	3	0.07	1.09	0.82	0.28	1.80	0.06	6.92
Genotypes	12	0.09	1.38	1.18	0.55	2.27	6.11	66.10
Years x Genotypes	12	0.04	1.16	1.08	0.48	1.92	9.41	85.95
Combined error	72	0.14	1.72	0.64	0.32	2.84	8.62	90.20

DF: Degrees of freedom

Table 10. Dry matter, digestible dry matter, crude protein and digestible protein values of the standard varieties and mutant lines in 2019, 2020 and two-year averages

Genotypes	Dry Matter (%)			Digestible Dry Matter (%)			Crude Protein (%)			Digestible Protein (%)		
	2019	2020	Ave.	2019	2020	Ave.	2019	2020	Ave.	2019	2020	Ave.
1 (TB603)	88.6	88.8	88.7	62.1	62.3	62.2	24.1	22.0	23.1	17.1	15.7	16.4
2 (TB605)	89.2	88.9	89.0	62.2	62.9	62.5	22.6	21.7	22.2	16.1	15.5	15.8
3 (TB802)	88.9	88.7	88.8	62.1	60.6	61.3	22.5	21.4	21.9	16.0	15.2	15.6
4 (TB805)	88.8	88.7	88.7	60.8	62.5	61.7	21.3	21.9	21.6	15.1	15.6	15.4
5 (TB806)	88.7	88.7	88.7	61.7	60.8	61.2	22.6	22.8	22.7	16.0	15.9	15.9
6 (TB1002)	89.3	88.9	89.1	60.9	61.7	61.3	21.6	22.0	21.8	15.3	15.6	15.4
7 (TB1005)	88.9	88.8	88.9	61.8	61.0	61.4	22.8	21.1	21.9	16.1	15.0	15.6
8 (TB1006)	88.8	88.6	88.7	62.0	61.7	61.9	22.0	22.7	22.3	15.7	16.0	15.8
9 (TB1007)	89.0	88.9	88.9	62.4	63.8	63.1	23.1	23.4	23.3	16.4	16.7	16.5
10 (TB1008)	88.8	88.9	88.9	60.0	62.0	61.0	22.0	22.3	22.2	15.5	15.8	15.7
11 (TB1009)	88.7	88.7	88.7	60.8	62.2	61.5	22.7	21.9	22.3	15.9	15.6	15.7
12 (Kansur)	89.0	89.1	89.1	61.7	62.5	62.1	23.0	22.3	22.6	16.2	15.8	16.0
13 (Tarm Beyazi-98)	89.1	88.7	88.9	61.9	61.7	61.8	22.4	24.0	23.2	15.9	16.8	16.4
Mean	88.9	88.8	88.8	61.6	62.0	61.8	22.5	22.3	22.4	15.9	15.8	15.9
LSD. $_{(0.05)}$	1.1	0.4	0.5	3.0	2.7	1.9	2.0	1.5	1.2	1.4	1.0	0.8
CV. (%)	0.6	0.2	0.4	2.3	2.0	2.1	4.0	3.0	3.6	4.2	2.9	3.6

LSD Least significant differences; CV (%) Coefficient of variation

The two-year average results indicated that dry matter (DM) content exhibited a range of values between 88.7 and 89.0%, with an average of 88.8%. The amount of digestible dry matter (DDM) changed in a range of 61.0 and 63.1% in the present study (Table 10). Furthermore, the literature indicates that DDM ratios range from 59.43 to 63.21% (Bingöl et al., 2007), 59.10 to 68.69% (Aksoy and Nursoy, 2010), 62.4% (Cacan and Yılmaz, 2015), and 56.0 to 65.5% (Bayar and Cacan, 2019). In this study crude protein (CP) rates ranged from 21.6 to 23.3% (Table 10). In addition in the literature CP rates were measured at 15.10-21.35% (Elvan, 2019); 23.1-26.8% (Bayar and Cacan, 2019); 17.66-24.89% (Guzelogulları and Albayrak, 2012); 22.0-27.0% (Eviz, 2016). Except for Elvan's (2019) earlier experiments support our findings. The concept of digestibility pertains to the capacity of feed materials for utilisation by livestock. A higher degree of digestibility indicates that a greater proportion of the ingested feed content can be processed by the digestive tract (Sari et al., 2018). Digestible protein (DP) ratio is lower than CP ratio. Consequently, DP ratio exhibited fluctuations between 15.4% and 16.5% in the present study (Table 10).

Table 11. Acid detergent fiber, neutral detergent fiber and relative feed values of the standard varieties and mutant lines in 2019, 2020 and two-year averages

Genotypes	Acid Detergent Fiber (%)			Neutral Detergent Fiber (%)			Relative Feed Values		
	2019	2020	Ave.	2019	2020	Ave.	2019	2020	Ave.
1 (TB603)	34.4	34.1	34.3	51.1	45.9	48.5	114.8	126.5	120.7
2 (TB605)	34.2	33.4	33.8	47.0	47.0	47.0	123.3	124.3	123.8
3 (TB802)	34.5	36.3	35.4	45.5	49.6	47.5	127.0	113.9	120.5
4 (TB805)	36.0	33.9	34.9	52.4	44.9	48.7	108.1	129.7	118.9
5 (TB806)	34.9	36.1	35.5	46.7	47.7	47.2	122.9	118.6	120.7
6 (TB1002)	36.0	34.9	35.4	49.8	46.8	48.3	113.8	122.9	118.3
7 (TB1005)	34.7	35.8	35.3	47.0	47.2	47.1	122.4	120.2	121.3
8 (TB1006)	34.5	34.9	34.7	47.6	47.6	47.6	121.2	120.6	120.9
9 (TB1007)	34.0	32.3	33.1	45.0	42.4	43.7	129.2	139.8	134.5
10 (TB1008)	37.1	34.5	35.8	49.3	44.8	47.1	113.9	129.0	121.4
11 (TB1009)	36.1	34.3	35.2	47.8	45.7	46.7	118.7	126.6	122.6
12 (Kansur)	34.9	33.9	34.4	46.1	47.2	46.6	125.1	123.8	124.4
13 (Tarm Beyazi-98)	34.7	34.9	34.8	47.5	45.5	46.5	121.3	127.1	124.2
Mean	35.1	34.6	34.8	47.9	46.3	47.1	120.1	124.8	122.5
LSD. $_{(0.05)}$	3.9	3.4	2.5	7.4	5.2	4.3	22.5	18.7	13.9
CV. (%)	5.1	4.6	4.8	7.1	5.1	6.2	8.6	6.9	7.8

LSD Least significant differences; CV (%) Coefficient of variation

The two-year average revealed that acid detergent fiber (ADF), neutral detergent fiber (NDF) and relative feed values (RFV) ranged from 33.1 to 35.5%, 46.5 to 48.7%, 8.3 to 134.5 respectively (Table 11). ADF ratios were found to be between 34.1% and 37.3% (Kusvuran et al., 2014), 34.1% (Cacan and Yılmaz, 2015), 30.01%-37.14% (Hashalıcı et al., 2017), 30.0%-42.3% (Bayar and Cacan, 2019), and 32.19%-39.87% (Elvan, 2019). Furthermore NDF ratios were identified at 38.3-45.0% (Kara, 2013), 40.7% (Cacan and Yılmaz, 2015), 39.05-46.79% (Hashalıcı et al., 2017), 37.2-7.5% (Bayar and Cacan, 2019), 45.87-54.46% (Elvan, 2019). RFV were determined to be 106-180 (Aksoy and Nursoy, 2010), 143.2 (Cacan and Yılmaz, 2015), 118.8 (Yılmaz et al., 2015), 112.0-163.8 (Bayar and Cacan, 2019), and 98.77 and 125.45 (Elvan, 2019).

Feeds are classified according to quality standards as either prime, 1st, 2nd, 3rd, 4th or 5th quality. The properties of crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), digestible dry matter (DDM) and relative feed value (RFV) can be employed to categorise feed according to its quality requirements (Cacan et al., 2015; Elvan, 2019). For this reason, the following parameters were determined in terms of forage quality: DM, DDM, CP, digestible protein (DP), ADF, NDF and RFV. The results of the current study are in agreement with those of previous trials (Hashalıcı et al., 2017; Bayar and Cacan, 2019; Ertekinet al., 2020). The results of the quality criteria analysis indicated that, while there was no significant difference between the genotypes in terms of mutagen applications, the TB1007 line demonstrated first-class feed value. The remaining genotypes exhibited a second-class quality of feed.

Hierarchical cluster analysis (HCA)

It is of significant importance to be aware of the similarities in agro-morphological traits associated with high forage yield in order to facilitate the development of effective breeding programs. The efficacy of breeding programs during the initial breeding period is contingent upon the accurate phenotype selection of numerous individual plants within the nursery plot.

Upon further examination, it becomes evident that there are two main groups (Figure 1). The groups are categorised according to the quantity of FY and HY. The group A is divided into two subgroups (A1 and A2) based on the observed differences in NPH, PH, and SN traits. Similarly, the group B is also classified into two subgroups (B1 and B2) due to the presence of distinct NPH, PH, SN, and SD traits. Upon analysis of the six traits in question, it was observed that there was a close relationship between NPH and PH, as well as between FY and HY. The TB805 line differed from other lines in terms of SD. The TB1006 line exhibited notable differences in PH and SN compared to other genotypes.

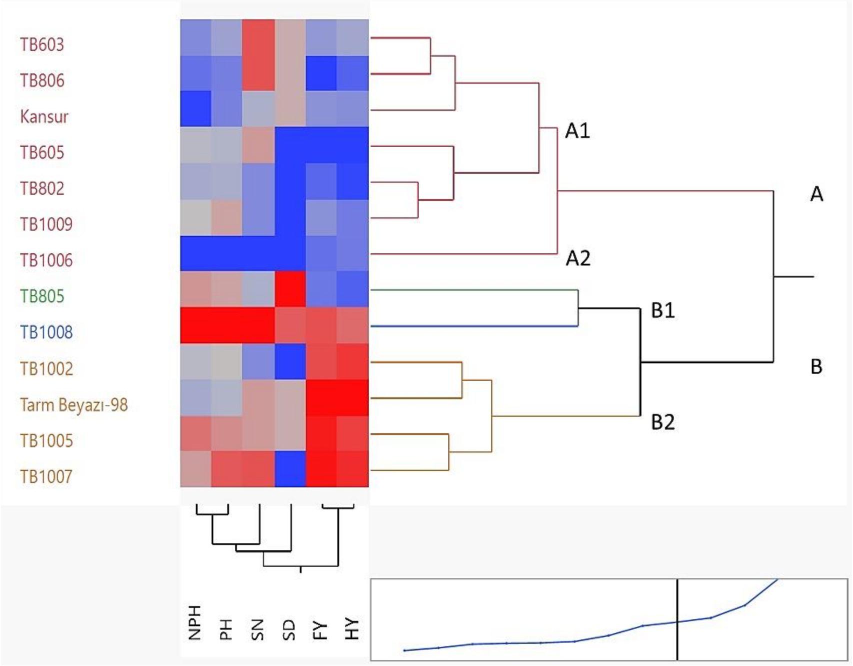

Figure 1. Dendrogram as determined by cluster analysis according to agro- morphological values and quality traits of genotypes

Principal component analysis (PCA)

Principal Component Analysis (PCA) was employed to ascertain the degree of correlation between traits and genotypes in the present study, as depicted on the biplot graph. The analysis also aimed to quantify the contribution of these traits and genotypes to the overall variation observed in the study data set (Figure 2).

Figure 2 presents the PCA results concerning the averages of the measured traits and genotypes. The mean value was calculated over two years. PCA1 and PCA2 collectively accounted for 57.1% and 23.3% of the total variation, respectively. When added together, they account for 80.4% of the total variation.

The examined traits were classified into six groups (Figure 2). The first group included NPH and PH, while the sixth included FY and HY. The TB1005 mutant line was located opposite other mutant lines such as TB605 and TB802. The TB1007, TB1005 and TB1002 mutant lines exhibited the highest data for both -FY and HY traits. The lowest yields were observed in the TB605, TB802, TB806 and TB1009 mutant lines. Additionally, the mutant line TB1006 exhibited the lowest SN.

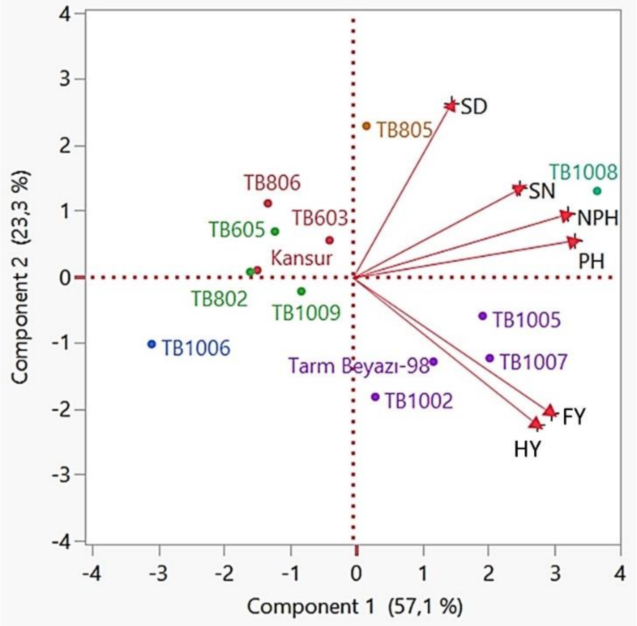

Figure 2. Biplot of principal component analysis on observed traits

4. CONCLUSIONS

A total of 13 line/variety sets, comprising 11 mutant lines and two control varieties, were subjected to an evaluation process to study the effects of mutations on morphological, agronomic and quality traits. Evaluation of the mutant lines revealed a range of effects on the traits examined, including both positive and negative results. A negative effect in the TB1006 mutant and a positive effect in the TB1008 mutant was observed in plant height. This has broadened the genetic base of Hungarian vetch. Nevertheless, the quality criteria remained largely unaltered. While the TB1002 and TB1005 mutant lines exhibited a yield advantage, the TB1007 mutant line demonstrated a yield and quality advantage. These three mutant lines are recommended for use in breeding programs, particularly in future crossing studies.

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CONFLICT OF INTEREST

All authors declare that they have no conflict of interest associated with this study.

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