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Agronomic performance, oil quality, and biodiesel potential of *Cephalaria syriaca* L. genotypes in Türkiye

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SUMMARY: Türkiye´s increasing shortage of edible oil and biodiesel feedstock underscores the need for alternative oilseed crops suited to different ecological conditions. This research investigated 17 promising genotypes and the registered cultivar Karahan of Pelemir (*Cephalaria syriaca* L.) across three distinct locations during the 2022–2024 growing seasons. Agronomic performance, oil and protein contents, fatty acid composition, and biodiesel quality parameters were assessed. Seed yield varied between 78 and 412 kg·da⁻¹, oil content ranged from 20 to 26%, and protein content from 14 to 24%, with significant genotype × environment interactions observed. The highest seed and oil yields were recorded in Bolu, where Loc-88 and Karahan showed superior productivity. Stability analysis identified A50, Loc-86, Loc-87, and Karahan as high-yielding and stable genotypes. Biodiesel properties, including iodine and acid values, met international standards, confirming Pelemir's suitability for biofuel production and its potential as a resilient oilseed crop for sustainable agriculture in Türkiye.

KEY-WORDS: *Biodiesel potential;* Cephalaria syriaca*; Genotype × environment interaction; Oil content; Pelemir; Seed yield.*

RESUMEN: ***Rendimiento agronómico, calidad del aceite y potencial para biodiésel de los genotipos* de Cephalaria syriaca L. *en Turquía.*** Turquía enfrenta un déficit creciente en aceites comestibles y materias primas para biodiésel, lo que resalta la necesidad urgente de cultivos oleaginosos alternativos adaptados a diversas condiciones ecológicas. Este estudio evaluó 17 genotipos prometedores y el cultivar registrado Karahan de Pelemir (*Cephalaria syriaca* L.) en tres localidades (Muş, Ankara y Bolu) durante las temporadas de cultivo 2022–2024. Se analizaron rasgos agronómicos, contenido de aceite y proteína, composición de ácidos grasos y propiedades seleccionadas de biodiésel. El rendimiento de semilla osciló entre 78 y 412 kg·da⁻¹, el contenido de aceite entre 20% y 26%, y el contenido de proteína entre 14% y 24%, con interacciones significativas genotipo × ambiente. Los rendimientos más altos de semilla y aceite se obtuvieron en Bolu, donde Loc-88 (412 kg·da⁻¹; 25% de aceite) y Karahan (377 kg·da⁻¹; 24% de aceite) mostraron un desempeño superior. El análisis de estabilidad identificó a los genotipos A50, Loc-86, Loc-87 y Karahan como de alto rendimiento y estables en todos los ambientes. Los parámetros de calidad del biodiésel, incluidos el índice de yodo (88–98 $gI_2 \cdot 100\ g^{-1}$) y el valor ácido (<2.6 mg $KOH \cdot g^{-1}$), cumplieron con los estándares internacionales, confirmando la idoneidad del aceite de Pelemir para la producción de biocombustibles. Estos hallazgos demuestran el potencial de Pelemir como cultivo oleaginoso tolerante al frío y la sequía y como un recurso genético valioso para expandir la materia prima de biodiésel en Turquía.

PALABRAS CLAVE: Cephalaria syriaca*; Contenido de aceite; Interacción genotipo × ambiente; Pelemir; Potencial para biodiésel; Rendimiento de semillas.*

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1. INTRODUCTION

With the increasing global energy demand and depletion of fossil fuel reserves, interest in sustainable and renewable energy sources is steadily growing (Çılğın 2020, Helal *et al.,* 2020). In this context, the investigation of new plant species for biodiesel production is crucial to achieving energy security and environmental sustainability goals. At the same time, determining the agronomic potential of new adaptive plant species is of great importance in increasing agricultural productivity and utilizing marginal lands in the face of changing climate conditions and an increasing global population (Güzel and Özyazıcı, 2021). Within this framework, the evaluation of drought-tolerant plants like *Cephalaria syriaca* L., which can adapt to different soil types, in terms of biodiesel production and agronomic performance, offers new opportunities for both energy independence and rural development strategies (Helal *et al.,* 2020; López *et al.,* 2021). *C. syriaca* is a plant that has industrial oil potential, particularly due to its epoxy acid content, along with its fragrant, greenish-yellow oil (Rahimi *et al.,* 2021). The oil obtained from the seeds of this plant is rich in unsaturated fatty acids such as oleic, linoleic, and linolenic acids, making it an attractive source for biodiesel production (Atabani *et al.,* 2013).

The genus *Cephalaria*, belonging to the Dipsacaceae family, is widely distributed among the flora of Türkiye and has traditionally been used for various purposes in some regions. For example, in some parts of Türkiye, the flour of *C. syriaca* is reported to have a dough-improving effect in bread-making. (Başar and Karaoğlu 2021). This demonstrates the interaction and potential of *C. syriaca* within local agricultural systems. However, a comprehensive examination of the agronomic performance of this species has been limited, especially in terms of its oil production potential within the framework of modern agricultural techniques. Studies on the adaptation abilities and yield characteristics of different genotypes are essential for understanding the plant's cultivability under various agro-ecological conditions (Galili *et al.,* 2018).

The value of vegetable oils in industrial applications largely depends on their fatty acid compositions (Fratianni *et al.,* 2021; Hatipoglu and Ak, 2023; Wu *et al.,* 2022). Research on the fatty acid content of some *Cephalaria* species has revealed that these plants contain important fatty acids such as myristic, palmitic, linoleic, alpha-linolenic, and oleic acids (Kırmızıgül *et al.* 2007). In particular, analyses of *Cephalaria* species collected from the southwestern Anatolia region of Türkiye show high proportions of unsaturated fatty acids like linoleic acid (33.02%) and alpha-linolenic acid (47.95%) (Kırmızıgül *et al.,* 2007). These findings suggest that *C. syriaca* seed oil may also contain valuable fatty acids in similar or different proportions. However, the specific oil quality of *C. syriaca* L. genotypes, the variations among different genotypes, and the effects of these variations on their industrial utilization potential have not been studied in detail. This gap in knowledge underscores the necessity for comprehensive research into the fatty acid profiles of diverse *C. syriaca* genotypes, which could reveal novel compositions which may be beneficial for various industrial applications. This comprehensive analysis would facilitate the identification of genotypes exhibiting superior oil quality for biodiesel production, thereby contributing to the development of *C. syriaca* as a viable energy crop (Berzuini *et al.,* 2024, Stanciu and Teacă 2024).

Ideal vegetable oils for biodiesel production should generally possess characteristics such as a suitable fatty acid profile, high oil yield, and sustainable production potential (Martínez *et al.,* 2014). While the biodiesel potential of various plant species has been investigated in Türkiye and globally (Çılğın, 2020), the potential of *C. syriaca* L. in this area remains largely unexplored. This situation creates a significant knowledge gap regarding whether this plant can be considered an alternative biodiesel source. To fill this gap, a detailed analysis of the differences in fatty acid composition among *C. syriaca* genotypes is of critical importance to determine whether they meet the suitability criteria for biodiesel production.

While limited research exists on the general potential of *C. syriaca* oil, a comprehensive, multi-location study evaluating the yield, oil quality variation across different genotypes, and the adherence of the resultant oil (or predicted FAME) to international biodiesel standards (e.g., TS EN 14214 or ASTM D6751) has not been previously conducted in Türkiye. This study, therefore, aims to fill this critical knowledge gap by comprehensively evaluating the agronomic performance, seed oil quality, and biodiesel production potential of *C. syriaca* L. genotypes in Türkiye. The data obtained will contribute to strategies for developing this species as a new industrial crop for biomass and biodiesel production and will form a scientific basis for efforts to achieve sustainable agriculture and biofuel goals in Türkiye.

2. MATERIALS AND METHODS

2.1. Material

The study was conducted across three distinct locations (Muş, Ankara, and Bolu) during the 2022–2024 growing seasons. In this study, seeds of 18 promising genotypes selected from a total of 69 genotypes collected from the natural flora of Türkiye were used, along with one promising line identified in previous studies. The registered cultivar Karahan, which was officially released in 2017, served as the standard. The Karahan cultivar was registered as an oilseed crop by the project team at the Field Crops Central Research Institute. In the official variety registration trials conducted by the Seed Registration and Certification Center, the Karahan cultivar, used as a standard, showed an oil content of 28.5% and a seed yield of 299 $kg \cdot da^{-1}$. The selection of these diverse genotypes, originating from various geographical and altitudinal regions across Türkiye, provides a solid basis for evaluating genotype-by-environment interactions and identifying adaptable lines for diverse agricultural systems.

2.1.1. Climatic conditions

The climatic data collected for the vegetation period indicate notable differences among the three study locations of Bolu, Muş, and Ankara. Total precipitation was highest in Muş (501.2 mm), followed by Bolu (479.0 mm), while Ankara received the lowest amount (329.9 mm). Average temperature values during the growing season also varied, with Muş exhibiting the highest mean temperature (15.7 °C), compared to Bolu (10.6 °C) and Ankara (10.0 °C). Relative humidity levels showed a similar pattern; Muş had the highest average humidity (71.9%), whereas Bolu presented slightly lower values (70.6%). These climatic distinctions suggest that Muş experiences relatively warmer and more humid conditions, while Ankara represents a drier and cooler environment during the crop-growing period.

2.1.2. Soil properties

Soil analyses from the experimental fields revealed textural and chemical variability among the locations. Ankara soils were classified as clay loam, whereas Muş and Bolu were categorized as clay. Soil salinity ranged from very low to moderate, with Bolu exhibiting the lowest salinity (0.02%) and Ankara the highest (0.81%). Organic matter content varied between 1.28% (Muş) and 2.32% (Bolu), indicating generally low to medium fertility levels. Lime content was notably higher in Ankara soils (29.1%) compared to Muş (0.26%) and Bolu (6.39%). Soil pH values were slightly alkaline across all sites, ranging narrowly from 7.40 to 7.51. Additionally, K_2O content showed significant variation, with Muş having the highest concentration (90.8 $kg \cdot da^{-1}$) and Bolu the lowest (47.73 $kg \cdot da^{-1}$).

2.2. Method

In the trial established in 2022, 17 promising genotypes which had previously demonstrated superior performance in terms of emergence rate, oil content, seed yield, and oil yield, both in their original location and in other locations, were sown in October 2023 in Muş, Ankara, and Bolu using a Randomized Complete Block Design (RCBD) with three replicates. Emergence observations were recorded before the onset of winter.

The experiments were set up with 25 cm between rows, 10 cm within rows, 5 m row length, and six rows per plot. Sowing was done manually at a depth of 3 - 4 cm, with 100 seeds per row and 600 seeds per plot. In spring, after the risk of frost had passed, thinning was carried out to leave 10 cm between plants within rows, resulting in 300 plants per plot. The total plot area was 1.25 m × 5 m = 6.25 m^2.

All observations, measurements, and weighings excluded the border rows and 50 cm from each end of the plot to avoid edge effects. No irrigation was applied during the trial period. Harvesting and threshing were performed manually.

In this study, the following traits of the genotypes were evaluated: plant height (cm), number of branches on the main stem, first branch height (cm), number of capitula per plant, thousand-seed weight, seed yield per decare ($kg·da^{-1}$), oil yield per decare ($kg·da^{-1}$), seed oil content, fatty acid composition, seed protein content, protein yield per decare ($kg·da^{-1}$), and certain biodiesel-related properties.

2.2.1. Oil content in seeds

Oil content analyses were conducted at Bolu Abant İzzet Baysal University, Faculty of Agriculture and Natural Sciences. Fatty acid composition analyses were performed at the Field Crops Central Research Institute. For the determination of oil content, seeds taken from each plot were ground and subsequently analyzed using the solvent (petroleum ether) extraction method in a Soxhlet apparatus (TS EN ISO 659).

2.2.2. Determination of fatty acid composition

To determine the oil composition, oils extracted from seeds obtained from each plot were used. For the analysis of fatty acid composition, 0.1 g of oil was mixed with 10 mL of *n*-hexane and shaken, followed by the addition of 0.5 mL of 2N methanolic KOH. The mixture was vortexed and allowed to stand for 30 minutes to ensure complete esterification. The upper phase was then collected and injected into a Shimadzu AOC-20i autoinjector.

Fatty acid composition was determined using a Shimadzu GC-2010 gas chromatograph (Japan) equipped with a flame ionization detector (FID) and a Teknokroma capillary column (100 m × 0.25 mm, 0.2 µm film thickness). Helium was used as the carrier gas at a flow rate of 0.94 mL/min, with a split ratio of 1:100. The operating temperatures were set as follows:

- Injector and detector: 250 °C
- Oven program: Initial isothermal hold at 140 °C for 5 min, followed by a temperature ramp of 4 °C/min up to 240 °C, where it was held for 20 min.

For fatty acid identification, Restek 35077 Food Industry FAME Mix (USA) was used as the reference standard.

2.2.3. Determination of grain protein content

Grain protein content analyses were conducted at Bolu Abant İzzet Baysal University, Faculty of Agriculture and Natural Sciences, using the Kjeldahl method.

2.2.4. Determination of biodiesel quality

The seeds obtained from the experiment established during the 2022–2023 vegetation period were used to set up the trials for the 2023–2024 vegetation period. However, the crude oil extracted from the seeds harvested from the 2023–2024 vegetation trials was insufficient in quantity for a comprehensive Fatty Acid Methyl Ester (FAME) property analysis. Due to limited seed quantities from the second-year experiments, biodiesel was not produced from all genotypes. Instead, oil samples were analyzed directly for water content, acid value, and iodine value. Full biodiesel production and transesterification analyses were only conducted for the Karahan cultivar, for which sufficient oil was available. Due to this limitation, the following crude oil quality parameters were analyzed with the available oil quantity. To support biodiesel suitability assessments, three key biodiesel-related parameters were evaluated:

- *Water content (ppm)*: Excess water negatively affects combustion efficiency and can cause microbial growth and corrosion in storage systems.
- *Acid value (mg $KOH·g^{-1}$)*: Indicates the amount of free fatty acids (FFA). High FFA levels hinder transesterification efficiency and may require pre-treatment.
- *Iodine value (g $I_2·100\ g^{-1}$)*: Reflects the degree of unsaturation; lower values are associated with better oxidative stability.

These parameters were selected because they are critical pre-indicators of the oil's suitability for biodiesel conversion. Water content is critical, as high levels can cause hydrolysis during storage and corrosion in engines. The Acid value (or free fatty acid content) is crucial as it dictates the necessity for pre-treatment before transesterification. The Iodine value serves as an indicator of the oil's degree of unsaturation, which correlates directly with oxidative stability; high values may necessitate antioxidants to meet quality standards.

The analyses were conducted in accordance with international standards at the laboratories of DB Agricultural Energy Industry and Trade Inc. Analytical methods: The test methods for water content (ppm), acid value (mg $KOH \cdot g^{-1}$), and iodine value ($gI_2 \cdot 100g^{-1}$) were performed as referenced in TS EN 14214. Additionally, key FAME physicochemical properties that could not be measured (including Kinematic Viscosity (KV), Cetane Number (CN), and Cold Filter Plugging Point (CFPP) were calculated using established fatty acid composition-based prediction models to provide a more complete assessment of the final fuel quality.

2.3. Statistical analysis and data evaluation

All measured data were subjected to analysis of variance (ANOVA) using a randomized complete block design (RCBD) with three replicates. Differences between means were compared using Duncan's Multiple Range Test (DMRT) at the 0.05 level of significance. Principal Component Analysis (PCA) was performed to visualize genotype and environment relationships based on multiple traits. Statistical calculations were made using the JMP Pro 11 software package (SAS Institute, NC, USA, 2013).

3. RESULTS

The findings obtained from field trials conducted in Muş, Ankara, and Bolu using the genotypes included in this study, as well as from laboratory analyses, are presented in Tables 4, 5, 6, and 7.

In the field trial established at the Muş location, where the Loc 59 and Loc 120 was not germinated, the highest seed yield was obtained from the Karahan variety with 242.5 $kg \cdot da^{-1}$, followed by the Loc-88 genotype with 223.9 $kg \cdot da^{-1}$. The highest oil contents were recorded as 25.1% for Loc-88, 24.4% for the Karahan variety, and the Loc-105 genotype, respectively. The highest oil yield was found for the Karahan variety (59.3 $kg \cdot da^{-1}$) and the Loc-88 genotype (56.3 $kg \cdot da^{-1}$), followed by the Loc-86 genotype with 51 $kg \cdot da^{-1}$. In the Muş location, no genotype surpassed the Karahan variety in terms of yield, oil content, or oil yield (Table 3).

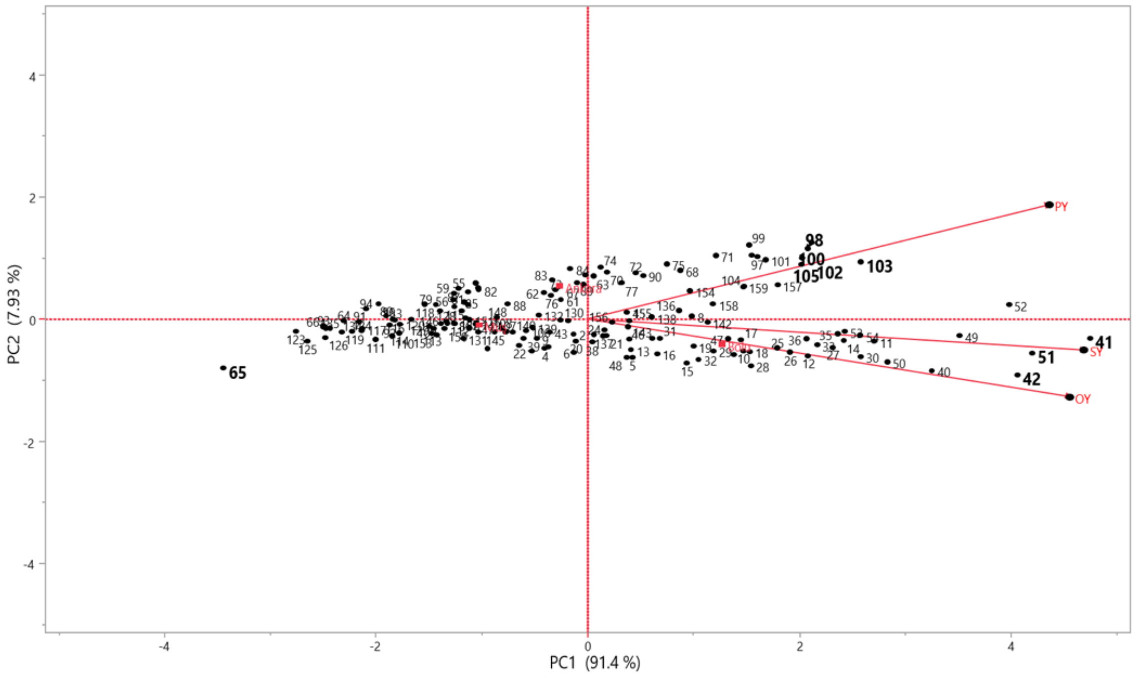
In the field trial established at the Ankara location, where Loc 59, Loc 88 and Loc 120 were not germinated, the highest seed yield was obtained from the Karahan variety with 258.0 $kg \cdot da^{-1}$, the A50 line with 273.0 $kg \cdot da^{-1}$, and the Loc-126 genotype with 269.9, followed by the Loc-86 genotype with 223.0 $kg \cdot da^{-1}$ (Table 4).

The highest oil content was obtained from the Karahan variety at 24.3%. The highest oil yield was found for the Karahan variety at 62.9 $kg \cdot da^{-1}$, followed by the A50 genotype with 54.2 $kg \cdot da^{-1}$ (Table 4). At the Ankara location, no genotype surpassed the Karahan variety in terms of yield, oil content, or oil yield.

In the field trial established at the Bolu location, where Loc 85 and Loc 105 were not germinated, the highest seed yield was obtained from the Loc-88 genotype with 412.1 $kg \cdot da^{-1}$, followed by the Karahan variety with 377.4 $kg \cdot da^{-1}$ and the A50 line with 343.2 $kg \cdot da^{-1}$. The highest oil content was obtained from the Loc-115 genotype at 25.1%, followed by Loc-86 at 24.3%, Loc-89 at 24.2%, and Karahan at 24.1%. The highest oil yield was found for Loc-88 with 98 $kg \cdot da^{-1}$ and the Karahan variety with 91.1 $kg \cdot da^{-1}$, followed by the A50 genotype with 80.1 $kg \cdot da^{-1}$ (Table 5). In the Bolu location, no genotype surpassed the Karahan variety in terms of yield, oil content, or oil yield.

The Figure 1 a presents a biplot based on principal component analysis (PCA), illustrating the relationships among seed yield (SY), oil yield (OY), and protein yield (PY), and their distribution across the pelemir genotypes evaluated in three different locations (Ankara, Bolu, Muş). The first two principal components (PC1 and PC2) explained a cumulative 98.79% of the total variation, with PC1 accounting for 91.5% and PC2 for 7.29%, indicating that most of the variability among genotypes can be effectively visualized in this two-dimensional space.

(a)



(b)

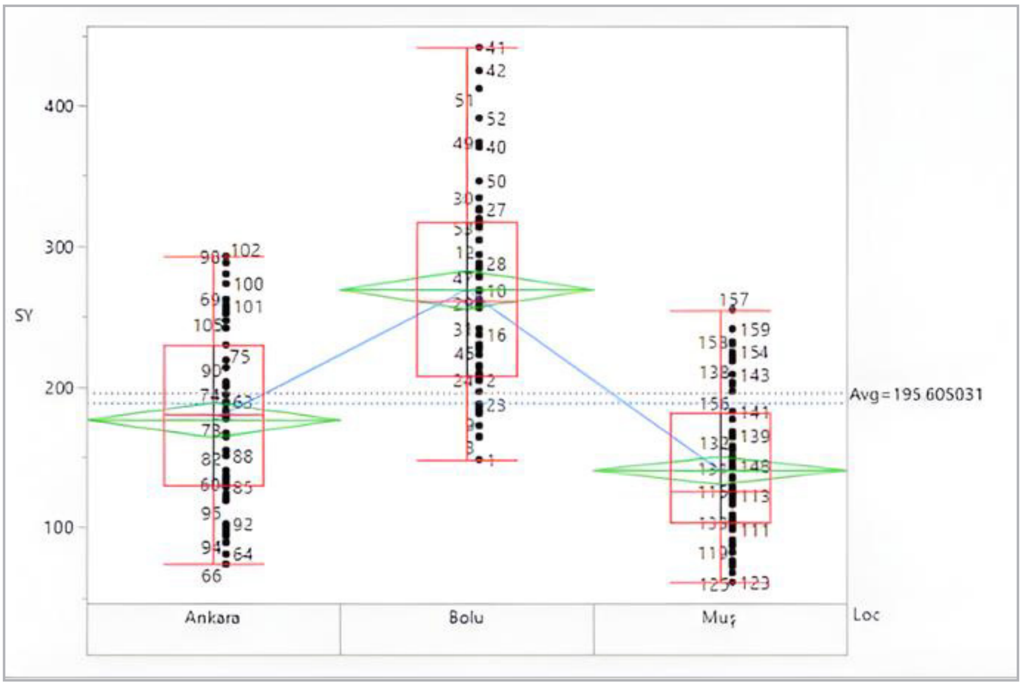


FIGURE 1. (a) Principal component biplot showing the distribution of *pelemir* genotypes and locations according to seed yield (SY), oil yield (OY), protein yield (PY) traits. **(b)** Variation in seed yield among pelemir genotypes evaluated at three locations: Ankara, Bolu, and Muş

The trait vectors (SY, OY, and PY) showed small angles between each other, suggesting strong positive correlations among these yield traits. The genotypes positioned in the direction of the SY and OY vectors (e.g., genotypes 41, 42, and 51) exhibited superior seed and oil yield potential, making them promising candidates for yield-oriented breeding programs. The genotypes located along the PY vector (e.g., 98, 100, 103, and 105) were found to be rich in protein yield. In contrast, genotypes such as 65 and those clustered on the left-hand side of the biplot, away from all three trait vectors, were associated with lower performance across these traits.

The Figure 1b illustrates the distribution of seed yield (SY, kg·da^{-1}) across the pelemir genotypes grown at the three different locations. The data reveal statistically significant differences in seed yield between locations, highlighting the influence of environmental conditions on genotype performance.

Bolu exhibited the highest average seed yield, with several genotypes (e.g., 41, 42, 49, and 51) producing yields exceeding 400 kg·da^{-1}, indicating a favorable agro-climatic environment for pelemir productivity. Ankara presented moderate seed yield performance, with most genotypes clustered around the overall mean (~195.6 kg·da^{-1}), though high-yielding genotypes such as 98 and 102 were observed. Muş, in contrast, demonstrated the lowest average seed yield, with most genotypes yielding below the general mean, suggesting less optimal growing conditions for pelemir in these locations.

In the stability analysis conducted for *C. syriaca* genotypes, genotypes 87, A50, and 86, along with the standard cultivar Karahan, were identified as both high-performing and stable in terms of seed yield (Figure 2a). Regarding oil yield, genotypes 86, 87, 88, A50, and the Karahan cultivar exhibited high yield potential and stability (Figure 2b). For protein yield, genotypes A50 and 86, as well as the Karahan cultivar, stood out as both productive and stable (Figure 2c).

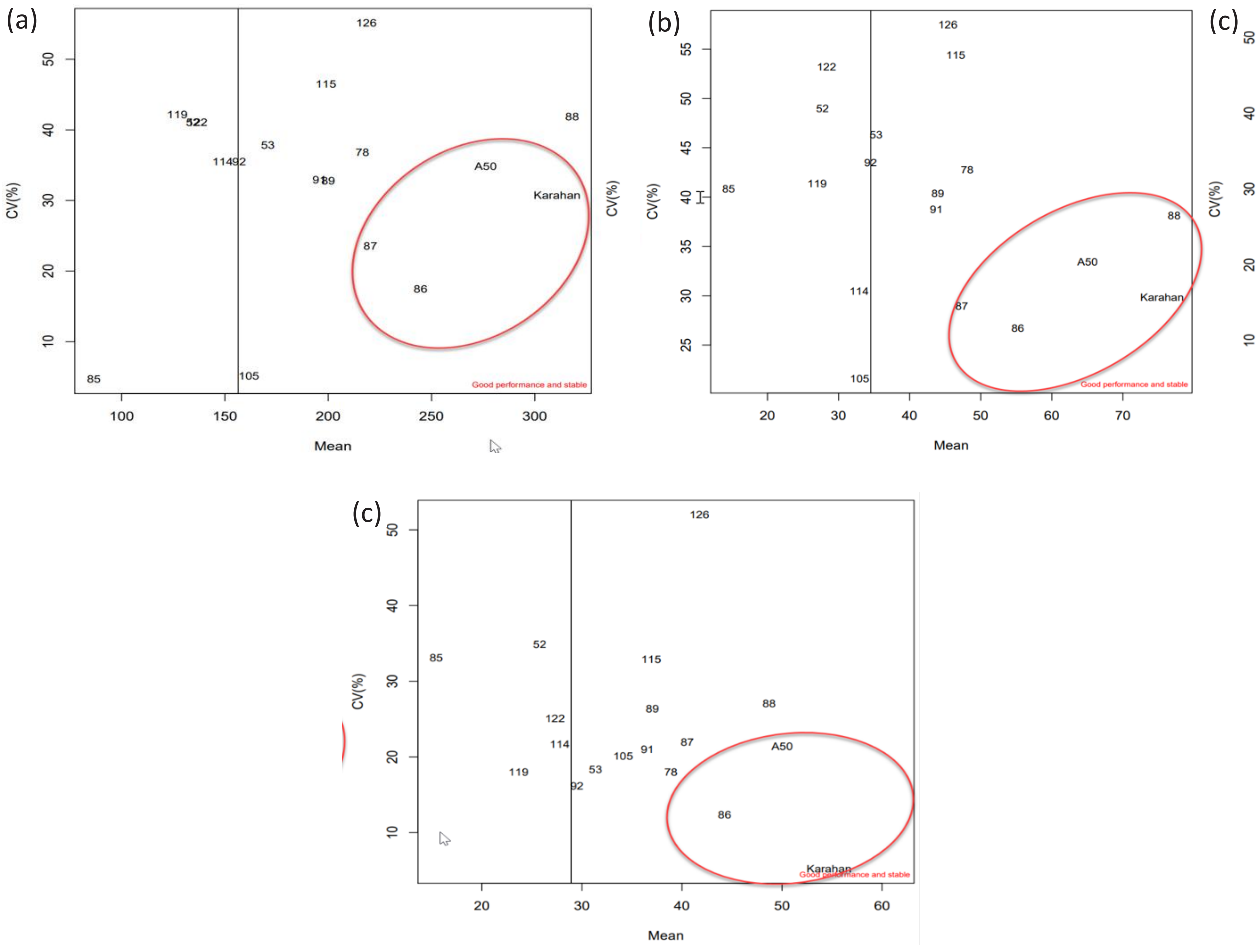


FIGURE 2. Seed yield (a), oil yield (b) and protein yield (c) stability analysis of pelemir genotypes across multiple environments

To determine the biodiesel quality of the prominent genotypes, only limited oil samples obtained from a portion of the seeds available from the 2023-2024 vegetation trials could be analyzed for water content (ppm), acid value (mg KOH g^{-1}), and iodine value (g iodine $100g^{-1}$). The findings are presented in Table 1.

As seen in Table 1, the free fatty acid values for the genotypes ranged between 0.99% and 2.62%, their moisture content varied from 575 ppm to 925 ppm, and their iodine values ranged from 88.81 g iodine/100g to 98.57 g iodine/100 g. Additionally, since sufficient seeds were available from the Karahan variety, an adequate amount of oil could be obtained, allowing for more comprehensive biodiesel analyses. The results related to the biodiesel properties of the Karahan variety are presented in Table 6.

TABLE 1. Location information of Pelemir genotypes and some oil parameters of prominent pelemir genotypes

No	Location No	Location	Altitude (m)	FFA (% Oleic)	water (ppm)	Iodine (g $I_2 \cdot 100\ g^{-1}$)
1	A50	Prominent line (Sivas kökenli)	1250	0.99	790	93.53
2	Loc-52	Kahramanmaraş/Afşin /Çomüdüz	1338	1.57	850	88.88
3	Loc-53	Kahramanmaraş/Afşin/Çoğulhan	1252	1.73	575	88.47
4	Loc-59	Malatya/Doğanşehir/Aydınlar	1099	1.65	621	89.30
5	Loc-78	Erzurum/İspir/Merkez	1297	1.21	760	93.66
6	Loc-85/4	Muş/Merkez/Şenoba	1281	-	-	-
7	Loc-86	Nevşehir/Avanos/Kalaba	1158	1.84	812	94.34
8	Loc-87	Yozgat/Boğazlıyan/Gövdecili	1039	2.62	870	97.37
9	Loc-88	Erzincan/Merkez/Mollaköy	1194	1.37	850	91.80
10	Loc-89	Erzincan/Merkez/Yaylabaşı	1211	1.46	811	93.81
11	Loc-91	Erzincan/Merkez/Tercan	1525	1.35	796	90.13
12	Loc-92	Bitlis/Ahlat/Adaksu	1970	1.17	810	96.71
13	Loc-105	Şanlıurfa/Siverek	754	-	-	-
14	Loc-114	Van/Özalp	2129	1.35	860	94.39
15	Loc-115	Van/İpekyolu/Bakraçlı	2108	1.06	785	88.81
16	Loc-119	Kahramanmaraş/Göksu/Yeşilkent	1570	1.85	745	90.79
17	Loc-120	Kayseri Sarız/Yeşilkent	1570	2.2	795	91.61
18	Loc-122	Bolu/Seben	839	1.58	810	92.80
19	Loc 126/1	Ağrı/ Çukurçayır village	1629	1.87	860	97.00

TABLE 2. Climatic data for the vegetation period of the locations and soil properties of the experimental fields

Months	Total Precipitation (mm)			Average Temperature (°C)			Average Relative Humidity (%)		
	Bolu	Muş	Ankara	Bolu	Muş	Ankara	Bolu	Muş	Ankara
October	44.8	80.2	4.4	13.7	21.4	13.6	73.8	73.9	67.4
November	125.6	15.8	60.4	10.1	15.4	8.8	74.0	77.4	68.6
December	69.9	61.2	28.6	6.4	8.0	4.6	78.9	85.7	83.7
January	82.8	45.4	60.1	2.9	3.5	1.9	78.8	86.6	76.8
February	19.8	49.4	12.9	6.0	7.1	4.6	68.9	81.2	70.4
March	31.6	155.6	40.2	7.1	11.1	6.1	70.3	76.5	73.2
April	20.7	50.2	26.9	14.3	21.6	14.3	58.8	59.2	68.5
May	77.1	35.0	68.5	13.4	20.8	13.7	71.3	62.6	69.9
June	6.7	8.4	27.9	21.9	32.5	22.4	60.8	43.9	69.4
Total	**479.0**	**501.2**	**329.9**	-	-	-	-	-	-
Average	-	-	-	**10.6**	**15.7**	**10.0**	**70.6**	**71.9**	**72.0**

Soil properties of the experimental field locations

Locations	Depth (cm)	Soil texture	Soil salinity (%)	Organic matter (%)	Lime (%)	pH	K_2O (kg·da^{-1})	P_2O_5 (kg·da^{-1})
Ankara	0-30	Clay Loam	0.81	1.55	29.1	7.51	74.33	2.93
Muş	0-30	Clay	0.40	1.28	0.26	7.40	90.8	10.2
Bolu	0-30	Clay	0.02	2.32	6.39	7.48	47.73	0.06

TABLE 3. Agronomic performance and quality parameters of *C. syriaca* genotypes at the Muş location, Türkiye, with mean separation based on Duncan's Multiple Range Test (DMRT)

Genotype	Plant height (cm)	First branch height (cm)	Number of branches (piece)	Number of heads per plant (piece)	Thousand seed weight (g)	Seed yield (kg·da^{-1})	Oil ratio (%)	Oil yield (kg·da^{-1})	Protein ratio (%)	Protein yield (kg·da^{-1})
Karahan	113.5 ab	64.6 ab	5.8 b-e	9.7 g-ı	14.6 c-e	242.5 a	24.4 ab	59.3 a	21.6 ab	52.5 a
A50	109.2 b-e	66.9 a	5.0 de	9.1 hı	14.6 b-e	207.5 c	23.8 bc	49.4 b	20.3 b-d	42.2 b
Loc 52	99.2 f-h	57.8 cd	5.4 c-e	10.7 f-ı	14.0 de	78.1 j	23.0 cd	17.9 ı	19.7 de	15.4 ı
Loc 53	93.2 hı	54.5 de	6.1 a-d	11.2 e-ı	14.1 de	124.4 e-g	23.4 cd	29.1 e	20 b-d	25.5 d-f
Loc 78	108.0 b-f	53.6 de	6.9 ab	14.1 c-e	15.6 a-c	162.4 d	23.2 cd	37.7 c	19.3 d-f	31.5 c
Loc 85	87.9 ı	47.1 fg	5.9 b-e	14.8 c	16.3 a	89.4 ıj	20.9 f	18.7 ı	21.2 bc	19.0 hı
Loc 86	113.1 bc	63.6 a-c	6.2 a-d	13.5 c-f	15.7 ab	217.3 bc	23.52 bc	51.0 b	18.35 fg	39.9 b
Loc 87	110.1 b-d	45.7 fg	7.4 a	24.0 a	14.2 de	168.6 d	23.7 bc	40.0 c	18.7 e-g	31.6 c
Loc 88	122.8 a	63.5 a-c	6.6 a-c	14.4 cd	14.5 c-e	223.9 b	25.1 a	56.3 a	17.5 g	39.3 b
Loc 89	103.6 d-g	64.6 ab	6.0 a-d	9.9 g-ı	14.3 de	134.2 ef	24.0 bc	32.31 d	19.5 d-f	26.1 d-f
Loc 91	99.7 f-h	59.0 b-d	6.1 a-d	11.1 f-ı	15 b-d	134.9 e	24.0 bc	32.3 d	20.5 b-d	27.7 c-e
Loc 92	98.6 f-h	53.9 de	5.3 c-e	11.3 e-ı	13.7 e	129.1 ef	23.5 bc	30.4 de	20.0 c-e	25.8 d-f
Loc 105	100.6 e-h	53.9 de	5.0 de	11.9 d-h	14.9 b-d	155.6 d	24.4 ab	38.0 c	18.8 e-g	29.2 cd
Loc 114	96.3 g-ı	43.8 g	5.7 b-e	19.5 b	14.5 de	108.4 gh	23.7 bc	25.7 fg	19.4 d-f	21.1 gh
Loc 115	101.9 d-h	57.7 cd	4.5 e	8.5 ı	14.4 de	118.5 f-h	23.4 bc	27.7 ef	19.6 d-f	23.3 fg
Loc 119	102.6 d-h	53.8 de	6.2 a-d	11.4 e-h	14.0 de	104.2 hı	22.4 de	23.1 gh	22.7 a	23.7 e-g
Loc 122	95.7 g-ı	46.2 fg	6.3 a-d	12.2 c-g	14.1 de	104.5 hı	21.2 f	22.1 h	21.1 bc	22.2 f-h
Loc 126	103.8 c-g	51.3 ef	6.3 a-d	14.3 cd	12.3 f	80.7 j	21.7 ef	17.5 ı	22.6 a	18.2 hı
CV	5.52	105.44	14.25	13.52		6.71	2.68	5.57	3.98	8.82
Significance	**	*	*	**	**	**	**	**	**	**

* : Means denoted by the same letter in the same column are not significantly different (5% level).
**: Means denoted by the same letter in the same column are not significantly different (1% level).
Note: All values presented are the mean of three replicates. Mean separation was performed using Duncan's Multiple Range Test (DMRT) at the 0.05 level of significance

TABLE 4. Agronomic performance and quality parameters of *C. syriaca* genotypes at the Ankara location, Türkiye, with mean separation based on Duncan's Multiple Range Test (DMRT)

Genotype	Plant height (cm)	Number of Branches (piece)	Number of heads per plant (piece)	Thousand seed Weight (g)	Seed yield (kg·da^{-1})	Oil ratio (%)	Oil yield (kg·da^{-1})	Protein ratio (%)	Protein yield (kg·da^{-1})
Karahan	104.4 ab	9.6 a-c	43.4 d-f	13.6 f-h	258.0 a	24.3 a	62.9 a	23.3 a-c	60.4 a
A50	108.8 a	10.6 a	48.0 b-e	13.6 f-h	273.0 a	19.9 cd	54.2 b	22.51 a-c	61.3 a
Loc 52	90.4 b-f	8.3 b-f	55.8 ab	13.3 gh	136.9 fg	16.03 ı	22.0 hı	23.5 ab	32.1 e-g
Loc 53	80.0 ef	8.1 b-f	51 b-e	11.1 j	143.2 fg	16.0 ı	22.9 hı	22.0 bc	31.4 fg
Loc 78	100.5 a-d	8.1 b-f	51.2 b-e	15.4 ab	179.1 de	19.3 c-e	34.7 e-g	22.1 a-c	39.7 de
Loc 85	75.3 f	7.8 c-f	52.8 b-d	15.2 b	83.7 j	20.7 bc	10.3 j	22.1 bc	11.8 ı
Loc 86	102.8 a-c	9.1 a-e	53.8 a-c	14.6 b-e	223.0 b	19.3 c-e	42.9 cd	22.6 a-c	50.4 b
Loc 87	100.0 a-d	9.3 a-d	52.1 b-e	14.3 c-f	220.3 bc	17.6 f-h	38.8 de	22.4 a-c	49.3 bc
Loc 89	92.3 a-f	9.9 ab	62.9 a	15.1 b-d	200.4 b-d	17.6 f-h	35.1 e-g	22.4 a-c	45 b-d
Loc 91	104.1 ab	7.8 c-f	36.8 f	15.1 bc	189.1 c-e	18.8 d-f	35.6 ef	22.1 bc	41.8 cd
Loc 92	84.6 d-f	7.26 f	38.0 f	13.9 e-g	120.4 g-ı	18.3 ef	21.9 hı	22.9 a-c	27.6 g
Loc 105	105.3 ab	7.6 d-f	43.0 ef	12.9 hı	167.4 ef	16.7 g-ı	27.9 gh	23.3 a-c	38.9 d-f
Loc 114	92.1 a-f	7.6 d-f	44.2 c-f	12.2 ı	129.4 gh	22.0 b	28.5 f-h	22.6 a-c	29.3 g
Loc 115	93.2 a-e	7.4 ef	51.6 b-e	16.2 a	178.0 de	20.4 c	36.4 de	22.9 a-c	41.0 d
Loc 119	93.6 a-e	7.73 d-f	37.8 f	13.6 f-h	88.5 ıj	20.4 c	18.1 ı	21.9 c	19.3 hı
Loc 122	85.6 c-f	7.4 ef	45.1 c-f	11.3 j	103.9 h-j	16.5 hı	17.3 ıj	23.6 a	24.5 gh
Loc 126	95.2 a-e	7.5 d-f	53.0 bc	14.3 d-f	269.9 a	18.2 e-g	49.1 bc	22.6 a-c	61.01 a
CV	10.99	13.02	11.9	3.63	11.05	4.96	13.28	4.13	12.4
Significance	*	*	**	**	**	**	**	*	**

* : Means denoted by the same letter in the same column are not significantly different (5% level).
**: Means denoted by the same letter in the same column are not significantly different (1% level).
Note: All values presented are the mean of three replicates. Mean separation was performed using Duncan's Multiple Range Test (DMRT) at the 0.05 level of significance

TABLE 5. Agronomic performance and quality parameters of *C. syriaca* genotypes at the Bolu location, Türkiye, with mean separation based on Duncan's Multiple Range Test (DMRT)

Genotype	First branch height (cm)	Number of branches (piece)	Number of heads per plant (piece)	Thousand seed weight (g)	Seed yield (kg·da^{-1})	Oil ratio (%)	Oil yield (kg·da^{-1})	Protein ratio (%)	Protein yield (kg·da^{-1})
Karahan	48.3 ab	5.6 cd	16.0 g-j	15 b-d	377.4 ab	24.1 a-c	91.1 a	14.9 d-g	56.5 a
A50	35.8 c-f	6.2 a-d	23.7 c-e	15.1 b-d	343.2 bc	23.4 b-e	80.1 b	16.6 ab	57.4 a
Loc 52	35.1 d-f	7.4 a	24.5 b-d	12.8 e	188.3 h	22.9 b-f	43.2 g-ı	15.7 b-d	29.7 fg
Loc 53	28.5 f	7.2 ab	28.0 bc	10.7 g	244.6 e-g	22.0 e-h	53.8 ef	15.1 c-g	37.0 c-f
Loc 59	36.5 b-f	6.46 a-c	24.7 b-d	10.7 g	208.0 gh	23.0 b-e	48.0 f-h	14.3 fg	29.8 fg
Loc 78	49.7 a	4.7 d	15.0 h-j	16.3 a	308.1 cd	23.3 b-e	71.8 b-d	14.7 d-g	45.3 bc
Loc 86	47.4 a-c	5.6 cd	14.5 h-j	15.1 b-d	293.8 d	24.3 ab	71.5 b-d	14.4 e-g	42.3 b-d
Loc 87	44.7 a-e	4.9 d	12.4 j	14.7 cd	272.2 de	23.1 b-e	63.1 de	14.9 d-g	40.5 b-e
Loc 88	46.5 a-d	6.2 a-d	20.0 e-g	15.7 ab	412.1 a	23.8 a-d	98.0 a	14.0 g	58.0 a
Loc 89	43.1 a-e	5.5 cd	22.5 d-f	15.5 a-c	265.2 d-f	24.2 ab	64.4 d	14.9 d-g	39.8 b-e
Loc 91	47.2 a-d	5.2 cd	18.8 f-h	15.6 ab	263.1 d-f	24.0 a-c	63.2 de	15.1 c-g	39.9 b-e
Loc 92	43.3 a-e	4.8 d	12.8 ıj	14.7 cd	220.9 f-h	23.1 b-e	51.1 fg	15.8 b-d	34.8 d-g
Loc 114	32.7 ef	5.9 a-d	21.6 d-f	12.6 ef	208.7 gh	21.5 f-h	44.3 f-ı	15.6 b-e	32.8 e-g
Loc 115	43.6 a-e	5.8 b-d	19.8 e-g	16.0 a	299.8 cd	25.1 a	75.2 bc	15.4 b-f	46.4 b
Loc 119	36.8 b-f	6.6 a-c	29.0 b	11.8 f	188.0 h	21.0 h	39.5 hı	14.8 d-g	27.8 g
Loc 120	37.6 a-f	7.2 ab	33.6 a	12.0 ef	172.4 h	21.2 gh	36.4 ı	16.2 a-c	28.0 fg
Loc 122	39.9 a-f	6.7 a-c	28.1 bc	11.8 f	201.3 gh	22.5 d-g	45.4 f-ı	17.4 a	35.0 d-g
Loc 126	39.5 a-f	5.4 cd	17.0 g-ı	14.6 d	304.7 cd	22.7 c-f	69.3 cd	15.0 c-g	46.0 bc
CV	17.94	15.26	12.8	3.69	11.09	3.83	9.66	5.03	13.66
Significance	*	*	**	**	**	**	**	**	**

* : Means denoted by the same letter in the same column are not significantly different (5% level).
**: Means denoted by the same letter in the same column are not significantly different (1% level).
Note: All values presented are the mean of three replicates. Mean separation was performed using Duncan's Multiple Range Test (DMRT) at the 0.05 level of significance

TABLE 6. Measured crude oil and predicted FAME properties of Karahan cultivar and compliance with international standards

Parameter	Measured Value (Karahan)	Unit	TS EN 14214 Standard	ASTM D6751 Standard	Compliance
Density (15 °C)	0.8844	g·cm^{-3}	0.860–0.900	Not specified	Meets EN 14214
Kinematic Viscosity (40 °C)	4.35	mm^2·s^{-1}	3.5–5.0	1.9–6.0	Meets both
Total Acid Number (TAN)	0.47	mg KOH·g^{-1}	≤ 0.50	≤ 0.50	Meets both
Cold Filter Plugging Point (CFPP)	–9	°C	Country-dependent (≤0°C typical)	Not specified	Acceptable
Iodine Value	93.17	g I_2·100 g^{-1}	≤ 120	Not regulated	Meets EN 14214
Water Content	200	ppm	≤ 500 ppm	≤ 500 ppm	Meets both
Monoacylglycerol (Monoolein)	0.41	%	≤ 0.80%	≤ 0.40% (total glycerin basis)	Meets EN 14214 (borderline ASTM)
Diacylglycerol (Diolein)	0.06	%	≤ 0.20%	Not specified	Meets
Triacylglycerol (Triolein)	0.01	%	≤ 0.20%	Not specified	Meets
Free Glycerol	0.01	%	≤ 0.02%	≤ 0.02%	Meets both

Cetane Number (CN) was predicted via a multiple linear regression approach relating FAME composition (carbon chain length, degree of unsaturation) to measured cetane numbers of pure methyl esters and biodiesel, following established compositional predictive models (Giakoumis and Sarakatsanis 2019, Lin and Wu 2022).

4. DISCUSSION

In the multi-location trials, several genotypes failed to germinate at specific sites, confirming that emergence is one of the most environmentally sensitive stages in *C. syriaca* L. The non-germination of genotypes such as Loc-59, Loc-88, and Loc-120 likely reflects limitations in seed vigor, dormancy behavior, or tolerance to early-season stresses such as low temperature, soil crusting, or moisture fluctuations.

These non-germination events have important implications for genetic stability and adaptability. Genotypes that repeatedly fail to emerge across environments show low ecological plasticity and weak establishment ability, making them unsuitable for broad adaptation. Although removing such lines narrows the genetic pool, it improves overall trial stability by retaining only genotypes capable of reliable emergence. Conversely, genotypes that germinate consistently across all sites exhibit stronger adaptive capacity and resilience. Thus, germination performance serves as a natural selection filter in multi-environment trials, aiding in the identification of stable and widely adaptable pelemir genotypes for cultivation in Türkiye.

In the second year of trials, 16 genotypes and one promising line selected from the first year were evaluated across Muş, Bolu, and Ankara. In Muş, plant height ranged from 88 to 122 cm, with first branch heights of 44–66 cm. Plants developed 5–7 lateral branches and produced 9–24 heads per plant, with 1000-seed weights of 12–16 g, seed yields of 78–243 $kg \cdot da^{-1}$, oil contents of 20–25%, and protein contents of 18–23%. In Bolu, plant heights were considerably shorter (29–50 cm), with 5–7 lateral branches, 17–28 heads per plant, 1000-seed weights of 11–16 g, seed yields of 172–412 $kg \cdot da^{-1}$, oil contents of 21–25%, and protein contents of 14–17%. In Ankara, plant heights ranged from 75 to 109 cm, first branch heights from 29 to 50 cm, with 7–11 lateral branches and 36–63 heads per plant. Here, 1000-seed weights were 11–16 g, seed yields 83–273 $kg \cdot da^{-1}$, oil contents 16–24%, and protein contents 22–24%.

Compared to the first year, the reduced variability across traits reflects the targeted selection of high-performing genotypes. The more uniform performance across locations suggests a genetic narrowing of the tested material, although significant site-specific effects remained. For instance, Bolu exhibited higher mean seed yields but lower protein contents, which may be linked to its cooler and more humid conditions, favoring seed filling but potentially diluting protein concentration due to a carbohydrate–protein trade-off. In contrast, Muş and Ankara, with more continental climates, produced higher protein contents at the expense of yield, consistent with environmental stress-induced protein accumulation patterns. These observations are consistent with previous research on other oilseed crops, where climatic conditions significantly modulate productive performance and oil composition. For example, studies on *Camelina sativa* have shown that environmental factors like temperature and precipitation directly influence seed yield and oil quality, with milder temperatures often correlating with higher oil content and specific fatty acid profiles (Zanetti *et al.,* 2017).

The agronomic trait ranges observed align closely with previous literature. Plant height values correspond to those reported by Katar *et al.* (2012), Arslan *et al.* (2014), and Subaşı and Başalma (2021), indicating stability of this trait under varying ecological conditions. Branch and head numbers fall within the ranges reported by Katar *et al.* (2012) and Arslan *et al.* (2014), suggesting that branching capacity is less environmentally plastic than yield or quality traits. The 1000-seed weights (11–16 g) were consistent with multiple prior studies (Arslan *et al.* 2014; Arslan *et al.,* 2012; Rahimi *et al.,* 2021), highlighting the strong genetic control of this trait. Seed yield values also matched literature ranges, including 164–174 $kg \cdot da^{-1}$ (Katar *et al*., 2012), 209–338 $kg \cdot da^{-1}$ (Arslan *et al.,* 2014), and 41–233 $kg \cdot da^{-1}$ (Sezgin *et al.,* 2017), but the wider upper range in Bolu (up to 412 $kg \cdot da^{-1}$) suggests a favorable genotype × environment interaction at this site.

Oil content values (20–26%) were comparable to previous findings (Arslan *et al.,* 2014; Katar *et al.,* 2012; Kavak and Baştürk 2020; Sezgin *et al.,* 2017), although slightly lower than the 28% reported by Murat Karaoğlu (2006). The lower oil content may be partly explained by genotype composition and partly by seasonal variation in temperature during seed maturation, as oil biosynthesis in oilseeds is highly temperature-sensitive. While most previous studies examined single genotypes or varieties, Kavak and Baştürk (2020) evaluated 11 genotypes from southern and eastern Türkiye, providing a partially comparable multi-genotype dataset. The highest oil and protein contents recorded in the present study—26 and 32%, respectively—suggest that there is untapped potential in the evaluated germplasm for breeding programs aimed at dual-purpose (high-oil and high-protein) varieties (Rani *et al.,* 2024). These findings underscore the importance of genotype-by-environment interactions in determining optimal cultivation regions and highlight the need for further research into the genetic mechanisms underlying oil and protein accumulation in *C. syriaca* (Ghidoli *et al.,* 2024). Ankara, with a more continental climate, produced higher protein contents at the expense of yield, consistent with environmental stress-induced protein accumulation patterns. This high protein content (14 to 24%) significantly increases the economic value of C. syriaca as a potential dual-purpose crop. While the high oil percentage serves as a promising source for biodiesel, the protein-rich seed meal remaining after oil extraction is highly valuable as a feed supplement, offering an additional income stream and contributing to sustainable crop production (Sarkar *et al.,* 2021). This dual utility underscores the importance of optimizing both oil and protein yields through strategic agricultural practices, thereby maximizing economic returns for farmers (Mohammad *et al.,* 2018).

In terms of genetic diversity, no prior studies have applied GBS-based SNP markers to pelemir. İşler *et al.* (2024) used SCoT markers to assess population structure, producing 229 bands (219 polymorphic) with 12 primers and reporting average values for effective allele number (Ne) of 1.45, gene diversity of 0.27, and Shannon's information index (I) of 0.42. The high polymorphism rate reported indicates considerable allelic variation, which could be harnessed for trait improvement. Integrating advanced genomic tools such as GBS with agronomic performance data could therefore accelerate the identification of marker–trait associations and facilitate the development of improved pelemir cultivars. Such an approach would enable precise genomic selection strategies, leveraging the extensive genetic diversity already documented in sesame germplasm and other oilseed crops (Berhe *et al.,* 2021; Ghidoli *et al.,* 2024). This advanced genomic understanding will allow for more targeted breeding, focusing on improving traits like yield components (e.g., number of branches, seeds per capsule) and oil quality, which are crucial for enhancing overall agronomic performance and biodiesel potential (Sabag *et al.,* 2021).

The analysis of crude *C. syriaca* L. oil, focusing on key properties such as acid value (0.47 mg $KOH \cdot g^{-1}$), water content (200 ppm), and iodine value (93.17 g $I_2 \cdot 100g^{-1}$), confirms its strong preliminary suitability for biodiesel production, as all measured values are well within the permissible limits set by major international standards such as TS EN 14214 and ASTM D6751. However, a major limitation of this study was the insufficient oil quantity to perform a complete physicochemical analysis on the actual produced FAME. This restriction inherently limits the definitive validity of the conclusions regarding the final applicability of *C. syriaca* L. oil as a direct biodiesel fuel. To overcome this, the use of fatty acid profile-based prediction models to estimate critical missing properties (KV, CN, CFPP) provided a highly reliable scientific basis for a comprehensive preliminary assessment, demonstrating that the potential FAME quality is likely to meet or exceed international standards.

5. CONCLUSIONS

This multi-location study demonstrated the significant potential of *C. syriaca* L. as a promising oilseed crop for the diverse agro-ecological conditions of Türkiye. Genotype × Environment interaction was a critical factor influencing yield, underscoring the necessity of location-specific genotype selection.

Agronomically, the genotypes A50, Loc-86, Loc-87, and the registered cultivar Karahan exhibited high-yielding performance and superior stability across the test environments. These genotypes are recommended for immediate commercial cultivation and large-scale biodiesel feedstock production due to their high yield and favorable oil characteristics.

From a quality perspective, the crude *C. syriaca* L. seed oil possesses highly favorable properties for use as a biodiesel feedstock. All measured crude oil parameters are well within the permissible limits specified by international biodiesel quality standards, specifically TS EN 14214 and ASTM D6751. Furthermore, the prediction-based estimations of critical FAME properties strongly indicate that the oil can be successfully processed into a high-quality biodiesel that meets or exceeds these standards. Therefore, *C. syriaca* L. is definitively recommended as a viable, alternative oil source for sustainable biofuel production in Türkiye.

Finally, the substantial protein content (14–24%) observed in the seeds suggests that this crop also holds significant dual-purpose value, with the protein-rich seed cake being a valuable co-product for the animal feed industry. Future research should prioritize polyploidy breeding and the selection of genotypes (such as those with the highest protein content) to maximize the dual-purpose potential of *C. syriaca* L.

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DECLARATION OF COMPETING INTEREST

The authors of this article declare that they have no financial, professional or personal conflicts of interest that could have inappropriately influenced this work.

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AUTHORSHIP CONTRIBUTION STATEMENT

Y. Arslan 01: Conceptualization, Methodology, Supervision. M. Yaşar 02: Data curation, Writing- Original draft preparation. S. Ünal 03: Visualization, Investigation. İ. Subaşı 04: Formal Analysis, Software V. Çiftçi 05: Software, Validation. T. Eryiğit 06: Writing- Writing- Original draft preparation, Reviewing and Editing. B. İşler 07: Data curation, Investigation.

ARTIFICIAL INTELLIGENCE USAGE

An AI-based language model, ChatGPT (OpenAI), was employed to enhance the accuracy and fluency of the manuscript translation. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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