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Identifying Candidate Parents for Opium Poppy Breeding by Integrated Multivariate Analysis

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ABSTRACT

Opium poppy (*Papaver somniferum* L.) is cultivated for pharmaceutical alkaloids and nutritional seeds, and cultivar improvement depends on identifying candidate parents that combine high yield potential with stable, heritable trait expression. Although multivariate methods have characterized genetic diversity in poppy germplasm, few studies link genetic parameter estimation to multivariate grouping to guide candidate parental choice before hybridization. This study evaluated 23 poppy hybrids and 2 standard cultivars over three growing seasons (2021-2023) in Ankara, Türkiye, integrating cluster analysis, principal component analysis, and genetic parameter estimation to determine candidate parents. 18 agronomic, phenological, and chemical traits were assessed. Cluster analysis resolved the material into 5 groups that differed in yield, morphology, and alkaloid profile. Substantial genetic variation was detected, and yield-related traits accounted for the largest share of total phenotypic variance (49%). Capsule yield, seed yield, capsule number, and 1000-seed weight combined high heritability with high genetic advance, indicating additive gene action and strong selection potential. Alkaloid traits showed greater environmental sensitivity (ECV 28-57%). Based on these findings, the hybrids were classified into high-yielding (H12, H15, H23), morphologically distinct (H18), and high-alkaloid (H21) hybrids as candidate parents for strategic crossing to maximize transgressive segregation. This integrated framework enables efficient parental selection for simultaneous improvement of yield and desired traits in poppy breeding programs.

1. INTRODUCTION

Opium poppy (*Papaver somniferum* L.), an annual crop of Mediterranean origin, is cultivated globally for its pharmaceutical alkaloids and nutritional seeds (Machado et al., 2025; Özgen & Burucu, 2025). Its cultivation is strictly regulated under international narcotic conventions, with Türkiye being one of the authorized producers (Baser & Arslan, 2014; Lande, 1962). The capsule is the primary site of alkaloid biosynthesis, accumulating pharmaceutically critical compounds such as morphine and noscapine (Labanca et al., 2018), while the seeds are nutritionally valuable due to their high oil (44-50%) and protein (22-24%) content (Kahraman-Yanardağ et al., 2024). Breeding objectives therefore vary by end-use; food-grade cultivars prioritize high seed yield and low alkaloid content, whereas pharmaceutical cultivars target high capsule yield and specific alkaloid profiles (Arora et al., 2025).

Considerable genetic variability exists within opium poppy, providing scope for improvement through selection; however, realizing stable genetic gains requires partitioning this variability into its heritable and environmental components (Cobb et al., 2019). Quantitative-genetic parameters, which are broad-sense heritability (h^2), genetic advance (GA), and the genotypic, phenotypic, and environmental coefficients of variation, indicate whether observed trait differences reflect additive gene action and are therefore responsive to candidate parental selection (Anand et al., 2023). Because such estimates are strongly modulated by climate and site variability, they must be derived under multi-environment evaluation to be reliable. Trait plasticity underscores this need; stigma number, for instance, generally ranges from 8 to 12 lobes but may vary from 4 to 18 depending on genotype and environment (Paniagua-Zambrana et al., 2025).

Multivariate methods, particularly hierarchical clustering and principal component analysis (PCA), are widely applied to characterize genetic diversity in poppy germplasm and to quantify the traits that drive variation (Kara & Baydar, 2018; Kumar et al., 2016; Mishra et al., 2016). These analyses effectively describe the structure of diversity and rank trait contributions; however, they classify hybrids based on phenotypic distance alone and do not establish whether the distinguishing traits are heritable and selectable. Consequently, such groupings reveal how hybrids differ, rather than which hybrids justify advancement as candidate parents. Few studies have coupled multivariate grouping with quantitative genetic parameter estimation to determine diversity structures in specific parental candidates before hybridization (Lahiri et al., 2018; Singh et al., 2023). This provides breeders with a systematic basis for selecting candidate parents that jointly offer high-yield potential, heritable trait expression, and morphological distinctness across environments.

The current study hypothesized that poppy hybrids exhibit distinct adaptive responses under heterogeneous multi-year environments and that integrating genetic variability estimates with multivariate analyses enables the identification of candidate parents possessing contrasting, heritable trait combinations. This approach would enable strategic crossing to maximize transgressive segregation. Therefore, the current study aimed to test this hypothesis by evaluating 23 poppy hybrids and 2 standard cultivars over three growing seasons in Ankara, Türkiye. 18 agronomic, phenological, and chemical traits were assessed using cluster analysis, PCA, and genetic parameter estimation. The objective was further extended to identify candidate parents with traits and high h^2 for key yield components, to understand a practical framework for candidate parental selection in poppy breeding.

2. MATERIALS and METHODS

Features of Experimental Area

The current study was conducted over three growing seasons (2021, 2022, 2023) at the experimental field of Ankara University, Faculty of Agriculture (39°55′ N, 32°51′ E, 891 m elevation), characterized by a hot-humid continental climate. Seasonal precipitation increased from 269.20 mm in 2021 to 371.40 mm in 2022 and remained relatively high at 366.90 mm in 2023, compared with the long-term average of 347.30 mm (1927–2024). Likewise, mean air temperature ranged from 9.52 °C in 2021 to 10.44 °C in 2022, with a value of 10.20 °C in 2023, consistently exceeding the long-term average of 8.70 °C. Mean relative humidity varied between 58.00% and 65.00% during 2021-2023 and was 65.40% in 2023, remaining comparable to the long-term average of 65.10%. Soil analysis indicated a silty-clay texture with low organic matter (0.88-0.90%), moderate electrical conductivity (0.16-0.22 dS m^{-1}), and slightly alkaline pH (7.65-7.83). The soil was rich in potassium (541-632 mg kg^{-1}) and had adequate phosphorus, copper, zinc, and manganese, but was deficient in nitrogen (0.032-0.041%). Nitrogen was supplied in two split applications, with 50 kg N ha^{-1} applied at sowing as DAP (18-46-0) and the remaining 50 kg N ha^{-1} applied at stem elongation as urea (46-0-0).

Genetic Materials and Agronomic Practices

23 poppy hybrids and 2 standard cultivars (ST1, ST2) were evaluated over three growing seasons (2021–2023) in Ankara, Türkiye. ST1 and ST2 were included as control plants because they belong to the morphine chemotype, characterized by morphine constituting up to 80% of the total alkaloid content, while noscapine represents up to 10-15%. The hybrids were selected based on prior performance for capsule yield, seed yield, oil content, and alkaloid profiles (e.g.,

morphine, noscapine). Each experimental plot comprised 4 rows, each 3 m in length and spaced 40 cm apart. Seeds were sown in early October at a seeding rate of 2.5 kg ha^{-1}. In early March, winter-damaged plants were removed and densely populated areas were manually thinned to achieve a uniform plant density of 13 plants m^{-1}. Supplementary irrigation was applied using a sprinkler irrigation system when natural precipitation was insufficient to meet crop water requirements, particularly during the rosette, stem elongation, and flowering stages. Irrigation was performed using sprinklers with an application rate of 1.5 m^3 h^{-1}, and the cumulative seasonal irrigation amount was 300 m^3 ha^{-1}. Based on soil analysis, 50 kg ha^{-1} each of N and P_2O_5 were applied: full P and half N at sowing, and the remaining N at stem elongation. Aphid control was carried out in mid-May using spirotetramat (Movento® SC 100, Bayer), whereas weeds were controlled manually during thinning. All agronomic traits were measured on 20 randomly selected plants from the two central rows of each plot to minimize border effects. Plant height was measured from the soil surface to the top of the main capsule. Capsule number, length, width, and stigma number were recorded from the main capsule. Seed and capsule yields per plant were determined by harvesting all capsules from sampled plants, then converted to kg ha^{-1}. Thousand-seed weight was measured after oven-drying at 103°C for 17 h following ISTA (2025) guidelines.

Extraction and Characterization of Alkaloids

Capsule samples obtained from each of four replicates were stored under ambient laboratory conditions and subsequently ground into a homogeneous fine powder using a laboratory grinder (TSM6A013B, Bosch, Germany) prior to alkaloid analysis. Alkaloid percentages were quantified by high-performance liquid chromatography (Shimadzu-HPLC) at the Bolvadin Alkaloid Factory (Afyonkarahisar, Türkiye). Capsule samples (5 g) were extracted in a 50 mL methanol-isopropanol solution for two hours at room temperature. The pH of the extracts was adjusted to 8.0 with 10% NH_4OH and partitioned with chloroform. The chloroform fractions were dried over anhydrous sodium sulfate (Na_2SO_4) and then concentrated to the final volume of the mobile phase. HPLC analysis was performed using a Waters system equipped with a Bondapak C18 column (30 cm × 3.9 mm, 10 µm) following the method of Barberi-Heyob et al. (1991) and Gümüşçü and Arslan (2008) with minor modifications. The mobile phase consisted of a phosphate buffer (pH 2.1-2.3), methanol linear gradient (95:5 to 60:40) at a flow rate of 1 mL min^{-1}. Detection was carried out with an HP-1040 M photodiode array detector at 190-400 nm. The analysis time was 15 min, and results were expressed as both individual and total alkaloid percentages (Valizadeh et al., 2017).

Analysis of Seed Fixed Oil Ratio

Seeds were finely ground to a homogeneous powder using a grinder (Bosch TSM6A013B, Germany). Precisely 5 g of the powdered material was weighed on an analytical balance and subjected to Soxhlet extraction with 50 mL of n-hexane at 70 °C for 2 hours. After extraction, the residual dry matter was weighed, and the fixed oil content was expressed as a percentage of the initial sample weight (AOAC, 2000).

Statistical Analysis

A balanced randomized complete block design with four replications was employed, with 23 poppy hybrids and 2 standard cultivars evaluated over three years. Normality and homogeneity of variances were verified using Shapiro-Wilk and Levene's tests, respectively. Analysis of variance (ANOVA) was conducted using the "*agricolae*" package (de Mendiburu & de Mendiburu, 2019) in *R*, with mean separation by Tukey's test ($p \leq 0.05$). Multivariate analyses included hierarchical clustering based on Euclidean distance and Ward's method, with the optimal number of clusters determined using average silhouette width according to Tibshirani and Walther (2005). PCA was performed to visualize trait relationships, and correlation matrices were generated using the "*metan*" and "*factoextra*" packages in *R* (Kassambara & Mundt, 2017; Olivoto & Lúcio, 2020). Prior to the multivariate analyses, all 18 traits were standardized to zero mean and unit variance (*z*-transformation) to remove the influence of differing measurement scales. Accordingly, the hierarchical clustering was based on Euclidean distances computed from the standardized data, and the PCA was derived from the correlation matrix. This ensured that variables measured on different units contributed comparably to both the distance structure and the ordination. Genetic parameters, genotypic variance (GV) and phenotypic variance (PV), coefficients of variations [Genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), environmental coefficient of variation (ECV)], broad-sense h^2, genetic advance (GA), and genetic advance as percent of mean (GAM), were estimated using the "*variability*" package in *R*.

$$Genotypic\ variance\ (GV)\ \left(\sigma_g^2\right) = MS_e - MS_{yg}/r\ y \quad (1)$$

$$Phenotypic\ variance\ (PV)\ \left(\sigma_p^2\right) = \sigma_g^2 + \sigma_e^2 \quad (2)$$

$$Broad\ sense\ heritability\ (h^2) = \frac{\sigma_g^2}{\sigma_p^2} \quad (3)$$

$$Genetic\ advance\ (GA) = h^2 \times \sigma_p^2 \times k \quad (4)$$

Where h^2 is heritability in broad sense, σ_p is phenotypic standard deviation of the trait, and k is the standard selection differential which is 2.06 at 5 per cent selection intensity.

$$Genotypic\ coefficient\ of\ variation\ (GCV) = \frac{\sqrt{GV}}{Grand\ mean} \times 100 \quad (5)$$

$$Phenotypic\ coefficient\ of\ variation\ (PCV) = \frac{\sqrt{PV}}{Grand\ mean} \times 100 \quad (6)$$

$$Environmental\ coefficient\ of\ variation\ (ECV) = \frac{\sqrt{EV}}{Grand\ mean} \times 100 \quad (7)$$

$$Genetic\ advance\ as\ percent\ of\ mean\ (GAM) = \frac{\sqrt{GA}}{Grand\ mean} \times 100 \quad (8)$$

3. RESULTS

ANOVA indicated significant (p≤0.05) differences in poppy hybrids' morphological characteristics for all the studied traits except capsule/seed (C/S) ratio and stigma number. Strong variations (p≤0.001) were observed for plant height, capsule number, capsule width, and capsule length (p≤0.01). Significant differences among years were observed for plant height ($p \leq 0.001$), capsule length ($p \leq 0.01$), and stigma number ($p \leq 0.05$). In contrast, year-to-year variation did not significantly influence capsule number, capsule width, and C/S ratio. Capsule yield (p≤0.001) and seed yield (p≤0.01) per plant showed highly significant variations among hybrids. Years affected only seed yield per plant (p≤0.05), whereas capsule yield was not. Capsule yield and seed yield per hectare were strongly influenced by poppy hybrids (p≤0.001) and years (p≤0.001). Morphine and noscapine content showed significant variation (p≤0.001). Year effects did not show significant variation in noscapine content and yield, whereas morphine content (p≤0.05) and yield (p≤0.01) were significantly affected. Significant differences in the year effect (p≤0.001) were observed for germination time, flowering time, and maturing time. For the phenological development, only flowering days showed significant variations (p≤0.01), while days to germinate and days to mature were statistically similar. The mean values of the opium poppy hybrids and years are presented in Table 1. The year effects of the studied traits and group labeling are presented in Figure 1.

Morphological characteristics

Among the hybrids, the maximum plant height (130.46 cm) was recorded for H21, followed by H12 (109.46 cm), H1 (108.18 cm), and H11 (106.93 cm). The maximum mean value was observed in 2022 (104.36 cm), followed by 2021 (100.63 cm), while the minimum occurred in 2023 (89.77 cm). The maximum capsule number was taken by H20 (7.45) along with H11 (7.33), H19 (7.16), and H16 (6.65). The mean capsule number was maximum in 2021 (5.25) and 2022 (5.22) but decreased notably in 2023 (4.45). The longest capsules were recorded for H6 (54.63 mm), H20 (54.38 mm), H11 (53.33 mm), and H18 (53.05 mm). ST2 had the maximum capsule width (48.68 mm), followed by H12 (45.63 mm), H18 (43.29 mm), and H14 (42.99 mm). H5 (1.21), H6 (1.20), H20 (1.18), and H9 (1.17) had the maximum C/S ratios. The maximum number of stigma was observed in line with H18 (18.74), H11 (11.83), H21 (11.19), and H10 (11.12).

Yield characteristics and related traits

The maximum capsule and seed yields per plant were observed in ST2 (4.47 g and 4.88 g), H15 (4.06 g and 4.27 g), H23 (4.03 and 4.64), and H12 (4.03 g and 4.47 g), respectively. The maximum capsule yield per hectare was obtained by ST2 (1491.90 kg), followed by H15 (1414.00 kg), H12 (1371.10 kg), and H23 (1277.40 kg). ST2 (1522.40 kg) also had the maximal seed yield per hectare, followed by H12 (1448.20 kg), H23 (1392.00 kg), and H15 (1391.10 kg). Seed yield per hectare followed a similar trend to capsule yield, showing a clear reduction in 2023. The maximum seed yield (1163.60 kg ha^{-1}) was obtained in 2021, whereas 2023 recorded a decrease of nearly 9.00%. Seed moisture content at harvest ranged from 8.71-8.94%, and after oven drying it decreased to 7.86-7.98%. However, differences in seed moisture content among hybrids were not statistically significant. H6, H15, and H17 exhibited the maximum 1000-seed weight (0.64 g), followed by H20 (0.63 g). In contrast, 1000-seed weight varied slightly but significantly among years, with the maximum mean value recorded in 2021 and the lowest in 2023.

Alkaloid contents and yields

ST1 (1.24%) had the highest morphine content among all poppy hybrids, followed by H5 (1.17%), H4 (1.14%), and H16 (1.04%). H11 (0.50%), H8 (0.47%), H10 (0.46%), and H15 (0.44%) had higher noscapine contents than the other poppy hybrids, in the same order. The highest morphine content (1.03%) and morphine yield (12.30 g ha^{-1}) were observed in 2021, followed by 2022. ST1 (16.40 g ha^{-1}) also had the maximum morphine yield per hectare. H22 (15.30 g ha^{-1}), H12 (12.10 g ha^{-1}), and H15 (12.00 g ha^{-1}) followed ST1 for morphine yield per hectare. Maximum noscapine yield per hectare was obtained by H15 (6.60 g ha^{-1}), followed by H10 (5.80 g ha^{-1}), H20 (4.70 g ha^{-1}), and H8 (4.50 g ha^{-1}). The overall mean noscapine yield of years ranged between 2.80–3.50 g ha^{-1}.

Phenological development

The earliest germination time (158 days) was observed for H4. H20 (163 days), H1 (167 days), ST1, and H16 (168 days) had the same germination time in poppy hybrids (Table 1). Germination duration varied drastically, from 10.51 days in 2021 to 20.08 days in 2023. Flowering time also differed significantly, being earliest in 2023 (196.16 days) and latest in 2022 (209.96 days). Similarly, the maturing time extended to 276.83 days in 2023, compared to only 244.14 days in 2021.

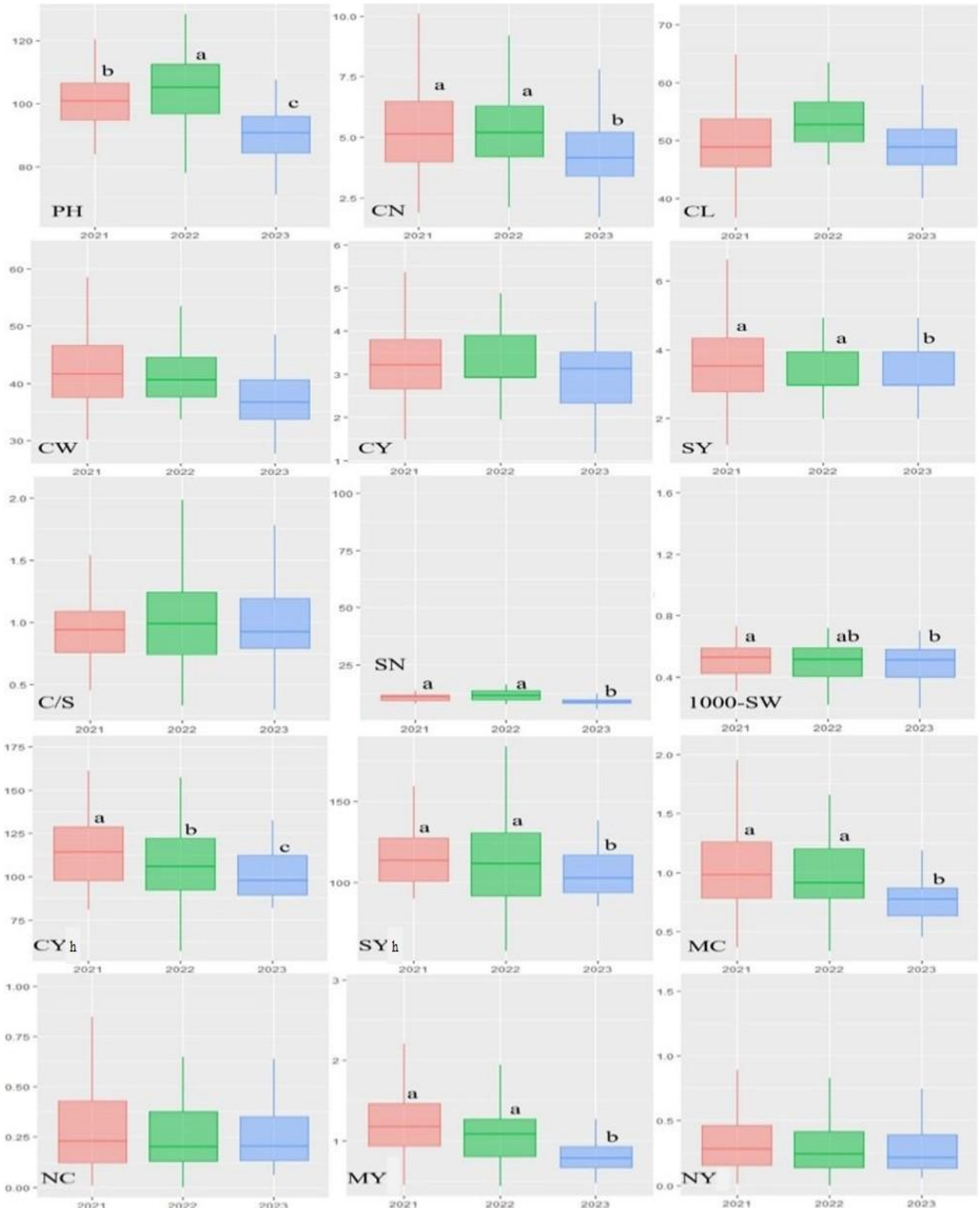

Figure 1. Year effects and group labeling of the studied traits (CN, Capsule Number; CL, Capsule Length; CW, Capsule Width; CY, Capsule Yield; C/S, Capsule/Seed; h, Hectare; MY, Morphine Yield per Hectare; NY, Noscapine Yield per Hectare; PH, Plant Height; SN, Stigma Number; SY, Seed Yield; 1000-SW, Seed Weight).

Table 1. Mean Agronomic Performance of the Traits on Poppy Hybrids

OPn	PH (cm)	CN (plant^{-1})	CL (mm)	CW (mm)	CY (g plant^{-1})	SY (g plant^{-1})	C/S	SN	CY (kg ha^{-1})
ST1	99.32 d-h	5.18 c-f	49.13 cde	38.68 e-j	3.60 bc	4.21 a-d	0.89	10.92	1255.10 cd
ST2	92.33 jk	4.81 d-g	52.20 a-d	48.68 a	4.47 a	4.88 a	0.95	10.61	1491.90 a
1	108.18 ab	4.17 f-k	50.56 a-e	41.40 cde	3.43 b-e	3.72 c-f	0.96	10.92	1066.10 fg
2	89.40 jk	3.36 jk	48.54 de	37.36 g-k	2.61 gh	2.83 fgh	1.02	10.61	964.40 h
3	88.08 k	3.28 k	48.22 de	35.09 k	2.37 h	2.60 h	0.97	10.61	850.80 ij
4	94.48 g-j	3.79 g-k	52.47 a-d	35.72 h-k	2.69 fgh	3.09 e-h	0.96	10.21	900.00 i
5	82.85 l	3.61 h-k	51.06 a-e	36.40 h-k	2.62 gh	2.68 gh	1.21	9.45	868.30 ij
6	93.58 h-k	3.60 ijk	54.63 a	35.50 ijk	3.10 c-g	3.09 e-h	1.20	9.81	1024.00 fgh
7	96.58 f-i	3.54 ijk	50.37 a-e	37.78 f-k	3.11 c-g	3.34 d-h	0.96	10.31	1048.60 fg
8	88.90 jk	4.46 e-j	50.12 b-e	35.18 jk	2.87 e-h	2.83 fgh	1.04	10.00	899.20 i
9	92.33 ijk	3.64 h-k	51.82 a-e	42.25 b-e	2.94 c-h	2.70 gh	1.17	10.65	1001.00 gh
10	105.15 a-d	4.72 d-h	50.93 a-e	41.03 c-f	3.61 bc	3.79 b-f	1.01	11.12	1234.50 cd
11	106.93 abc	7.33 a	53.33 abc	41.74 cde	3.04 c-h	3.27 e-h	1.00	11.83	807.90 j
12	109.46 a	4.79 d-g	51.15 a-e	45.63 b	4.03 ab	4.47 abc	0.94	10.12	1371.10 b
13	99.66 d-g	5.59 b-e	49.69 cde	39.12 d-h	3.50 b-e	3.59 c-g	1.02	10.52	1204.30 d
14	102.23 b-f	5.18 c-f	48.24 de	42.99 bc	2.94 c-h	3.21 e-h	1.08	10.25	1021.60 fgh
15	96.79 f-i	4.99 c-f	48.37 de	41.81 cde	4.06 ab	4.27 abc	0.98	10.62	1414.00 b
16	100.63 def	6.65 ab	49.88 cde	41.68 cde	2.91 d-h	3.18 e-h	0.95	11.02	1037.50 fg
17	101.12 c-f	4.56 d-i	50.58 a-e	39.16 d-h	2.75 fgh	3.11 e-h	0.88	11.11	1020.80 fgh
18	103.09 b-e	5.71 bcd	53.05 abc	43.29 bc	3.06 c-g	3.26 e-h	0.96	18.74	1085.10 e
19	104.69 a-e	7.16 a	50.28 a-e	41.20 c-f	2.45 gh	2.91 fgh	0.86	10.64	871.50 ij
20	103.45 b-e	7.45 a	54.38 ab	40.64 c-g	3.33 c-f	2.97 fgh	1.18	10.37	1062.10 fg
21	130.46 b-e	6.10 bc	51.48 a-e	42.70 bcd	3.43 b-e	3.55 c-h	0.96	11.19	1138.40 e
22	96.98 f-i	5.63 bcd	48.39 de	39.99 e-i	3.59 bcd	4.02 a-e	0.90	10.13	1233.70 cd
23	98.70 e-h	5.03 c-f	47.68 e	41.37 cde	4.03 ab	4.64 ab	0.92	9.83	1277.40 c
				Years					
2021	100.63 b	5.25 a	50.09	42.09	3.27	3.27 a	0.98	11.69 a	1155.00 a
2022	104.36 a	5.22 a	52.94	41.21	3.37	3.37 a	1.02	11.71 a	1089.00 b
2023	89.77 c	4.45 b	48.96	37.31	3.03	3.03 b	1.00	9.10 b	1013.90 c
				***Analysis of variance* (F-values)**					
Year	159.96***	19.58**	1.07	2.45	2.77	7.57*	0.29	7.43*	43.71***
OPn	13.69***	13.16***	2.26**	9.36***	7.36***	5.20**	0.93	1.17	72.28***
Y*OPN	0.70	0.99	0.30	0.35	0.69	0.63	0.13	0.98	6.40***

CN: Capsule number, CL: Capsule length, CW: Capsule width, CY: Capsule yield, C/S: Capsule/seed, PH: Plant height, SN: Stigma number, SY: Seed yield, OPn: Opium poppy number, 1000-SW: 1000 Seed weight, *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$.

Table 1. Continued

OPn	SY (kg ha^{-1})	1000-SW (g)	MC (%)	NC (%)	MY$_h$ (g ha^{-1})	NY$_h$ (g ha^{-1})	GER (days)	FLO (days)	MAT (days)
ST1	1339.00 c	0.51 efg	1.24 a	0.25 c-g	16.4 a	3.1 d-i	13.92	199.08 cd	258.83
ST2	1522.40 a	0.49 e-h	0.58 e	0.24 c-h	9.0 e-h	3.8 c-g	15.17	203.33 bcd	259.50
1	1107.40 ef	0.36 jkl	0.98 bcd	0.12 gh	11.2 b-e	1.4 k	14.17	200.25 a-d	256.92
2	989.30 g	0.47 ghi	0.81 cd	0.15 fgh	8.2 fgh	1.5 jk	13.17	202.25 ab	242.00
3	891.30 ij	0.48 fgh	0.89 cd	0.17 e-h	7.9 h	1.5 jk	14.17	204.00 ab	258.92
4	972.80 gh	0.55 c-f	1.14 ab	0.20 d-h	10.7 b-f	1.9 ijk	14.08	203.92 ab	256.92
5	908.40 hij	0.51 efg	1.17 ab	0.16 fgh	10.7 b-g	1.4 k	14.58	201.08 a-d	259.42
6	1007.10 g	0.64 a	0.89 cd	0.18 d-h	9.7 b-h	2.0 ijk	14.67	202.33 a-d	260.08
7	1073.10 f	0.61 abc	0.91 cd	0.15 fgh	10.1 b-h	1.5 jk	14.17	200.33 bcd	257.00
8	951.60 ghi	0.59 a-d	0.87 cd	0.47 a	8.1 fgh	4.5 cd	14.83	203.08 abc	259.42
9	988.30 g	0.57 b-e	0.87 cd	0.18 d-h	9.2 d-h	1.9 ijk	14.83	202.08 a-d	256.42
10	1250.10 d	0.28 m	0.79 d	0.46 ab	10.3 b-h	5.8 ab	13.92	202.83 a-d	257.17
11	844.80 j	0.41 ijk	0.99 bcd	0.50 a	8.4 fgh	4.3 cde	13.92	203.67 ab	258.17
12	1448.20 b	0.33 lm	0.83 cd	0.19 d-h	12.1 b	2.7 f-k	15.17	200.00 bcd	257.92
13	1233.80 d	0.42 hij	0.88 cd	0.30 cd	11.1 b-e	3.8 c-h	15.00	203.08 abc	257.75
14	1077.20 f	0.59 a-d	0.82 cd	0.29 cde	8.7 e-h	3.2 d-i	15.25	202.50 a-d	257.00
15	1391.10 bc	0.64 a	0.82 cd	0.44 ab	12.0 bc	6.6 a	15.67	203.83 ab	257.67
16	1118.00 ef	0.55 c-f	1.04 abc	0.27 c-f	11.3 b-e	2.9 e-j	14.00	204.67 a	259.50
17	1075.60 f	0.64 a	0.91 cd	0.22 d-h	9.9 b-h	2.4 h-k	14.67	199.08 cd	257.17
18	1122.90 ef	0.35 kl	0.83 cd	0.35 bc	9.4 c-h	4.1 c-f	14.67	202.83 a-d	260.92
19	912.50 hij	0.53 d-g	0.88 cd	0.35 bc	8.0 gh	3.2 d-i	14.92	201.58 a-d	257.17
20	993.20 g	0.63 ab	0.97 bcd	0.42 ab	10.8 b-f	4.7 bc	13.58	204.58 a	258.17
21	1166.10 e	0.38 jkl	0.99 bcd	0.35 bc	11.7 bcd	4.2 c-f	15.00	201.58 a-d	259.50
22	1268.80 d	0.55 c-f	0.16 ab	0.19 d-h	15.3 a	2.4 g-k	14.50	203.92 ab	260.08
23	1392.00 bc	0.52 d-g	0.90 cd	0.12 h	11.9 bc	1.6 jk	14.75	199.00 d	258.50
					Years				
2021	1163.60 a	0.53 a	1.03 a	0.29	12.3 a	3.5	10.51 c	200.47 b	244.14 c
2022	1138.20 a	0.50 ab	0.98 a	0.26	11.0 a	2.9	12.96 b	209.96 a	252.08 b
2023	1063.50 b	0.48 b	0.77 b	0.26	8.2 b	2.8	20.08 a	196.16 c	276.83 a
					***Analysis of variance* (F-values)**				
Year	45.84***	5.16*	6.17*	0.71	13.64**	2.58	209.46***	209.02***	176.16***
OPn	70.37***	21.19***	4.72***	9.84***	6.88***	10.33***	0.97	2.18**	0.78
Y:OPn	8.88***	1.07	0.92	0.87**	1.06	2.01***	1.50*	0.99	1.10

GER: Germination time, FLO: Flowering time, SY: Seed yield, MAT: Maturing time, MC: Morphine content, MYh: Morphine yield per hectare, NC: Noscapine content, NYh: Noscapine yield per hectare, OPn: Opium poppy number, 1000-SW: Seed weight, *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$.

Clustering of the Poppy Hybrids and Groups Differentiation

Cluster analysis based on 18 agronomic and chemical traits classified the 23 hybrids into 5 distinct groups (Figure 2, Table 2). According to the data analysis, all 23 poppy hybrids were grouped into 5 major clusters. Cluster 1 contained 9 hybrids including the top-yielding commercial varieties (ST1, ST2) and hybrids such as H12, H15, and H23, which were characterized by high capsule and seed yields (mean 1283.10 and 1328.10 kg ha^{-1}, respectively). Cluster 4 (H18) was distinguished by the highest stigma number (18.74) and capsule width (43.29 mm). Cluster 5 (H21) exhibited the tallest plants and high alkaloid content. Clusters 2 and 3 comprised lower-yielding hybrids, with Cluster 2 (H2) showing the lowest values for most traits. The cluster means for all traits are summarized in Table 2.

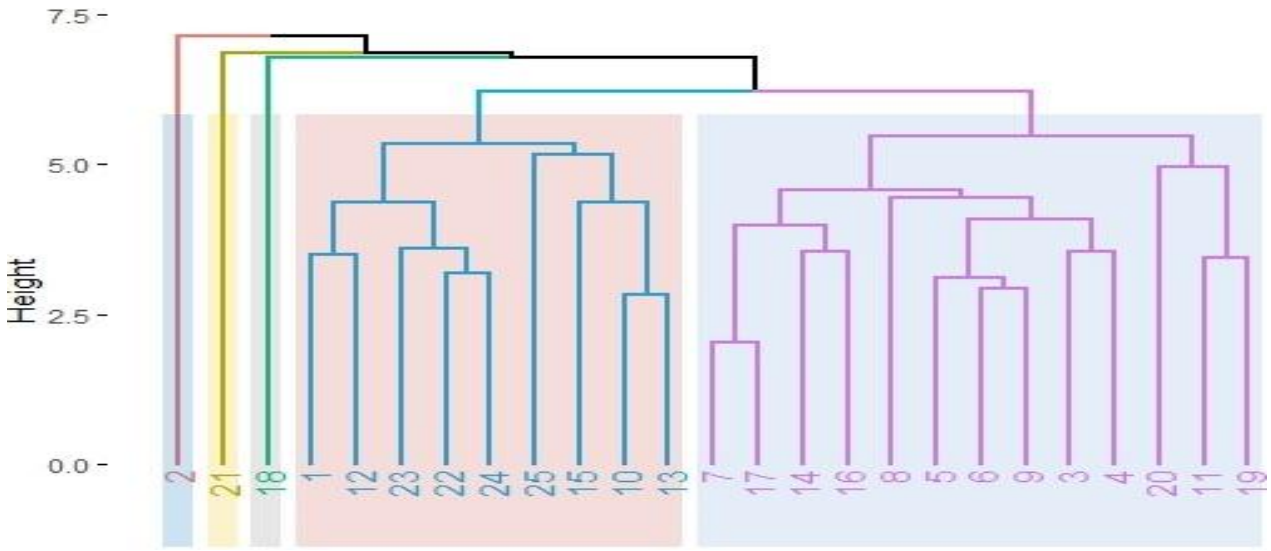

Figure 2. Cluster dendrogram (24, ST1; 25, ST2).

Table 2. Cluster groups and their cluster means

Cluster Groups	Cluster Groups	
	Number of poppy hybrid(s)	Member(s)
Cluster 1	9	1, 10, 12, 15, 13, 22, 23, ST1, ST2
Cluster 2	1	2
Cluster 3	13	3, 4, 5, 6, 7, 8, 9, 11, 14, 16, 17, 19, 20
Cluster 4	1	18
Cluster 5	1	21

	Cluster Groups Means								
	PH (cm)	CN (plant^{-1})	CL (mm)	CW (mm)	CY (g plant^{-1})	SY (g plant^{-1})	C/S	SN	CY (kg ha^{-1})
Cluster 1	100.50	4.99	47.79	41.86	3.81	4.18	0.95	10.45	1283.10
Cluster 2	89.36	3.36	48.54	37.35	2.61	2.83	1.02	10.61	964.40
Cluster 3	96.60	4.94	51.18	38.85	2.85	3.00	1.04	10.48	954.90
Cluster 4	103.09	5.71	53.05	43.29	3.06	3.26	0.96	18.74	1085.10
Cluster 5	103.46	6.11	51.49	42.70	3.43	3.55	0.96	11.19	1138.40
	SY (kg ha^{-1})	1000-SW (g)	MC (%)	NC (%)	MY_h (g ha^{-1})	NY_h (g ha^{-1})	GER (days)	FLO (days)	MAT (days)
Cluster 1	1328.10	0.46	0.91	0.26	12.10	3.50	14.65	201.70	258.26
Cluster 2	989.10	0.47	0.81	0.15	8.20	1.50	15.17	202.25	242.00
Cluster 3	985.70	0.56	0.95	0.27	9.50	2.70	14.33	202.53	258.10
Cluster 4	1122.90	0.35	0.83	0.35	9.40	4.10	14.67	202.83	260.92
Cluster 5	1166.10	0.38	0.99	0.35	11.70	4.20	15.00	201.58	259.50

CN: Capsule number, CL: Capsule length, CW: Capsule width, CY: Capsule yield, C/S: Capsule/seed, GER: Germination time, FLO: Flowering time, PH: Plant height, SN: Stigma number, SY: Seed yield, MAT: Maturing time, MC: Morphine content, MYh: Morphine yield per hectare, NC: Noscapine content, NYh: Noscapine yield per hectare, 1000-SW: Seed weight, 24:ST1, 25:ST2.

Multivariate Trait Variability by Principal Component Analysis (PCA)

Correlation coefficients of the studied traits were presented in Figure 3. Insignificant relationships were excluded in Figure 3.

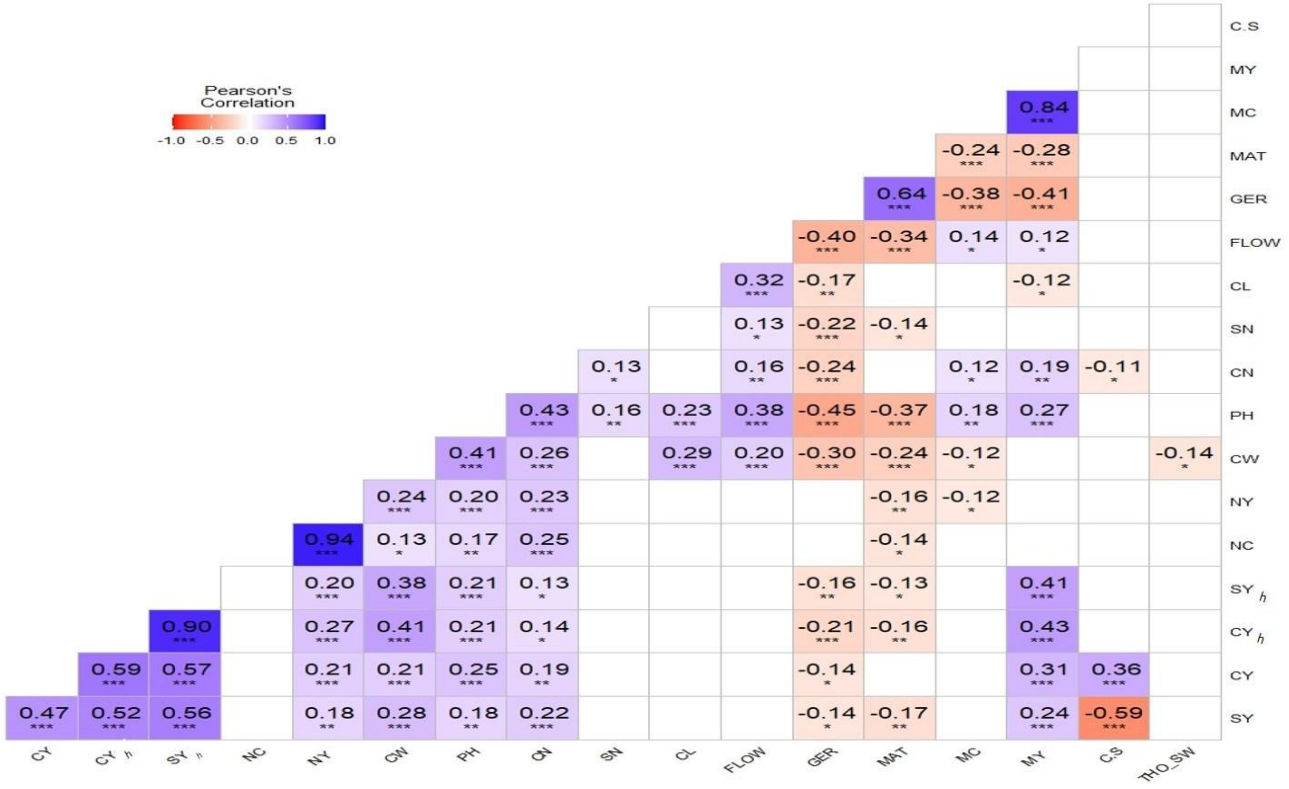

Figure 3. Correlation matrix of the traits (*, $p \leq 0.05$; **, $p \leq 0.01$; ***; $p \leq 0.001$).

PCA reduced the 18 traits to six dimensions, explaining 80.93% of the total variance, with the first two dimensions (Dim1 and Dim2) accounting for 49% (eigenvalues 5.50 and 3.32; Figure 4a). The top ten contributors of Dim 1 and 2 were: seed yield (plant^{-1} and kg ha^{-1}), capsule yield (plant^{-1} and kg ha^{-1}), noscapine content and yield, capsule number, capsule width, morphine yield, and plant height (Figure 4c). A narrow angle among these traits in the correlation circle (Figure 4d) indicates positive inter-trait relationships. The PCA biplot (Figure 4b) confirmed the distinctiveness of hybrids from different clusters: high-yielding Cluster 1 hybrids (H12, H15, and H23) aligned closely with yield-related traits, whereas H18 (Cluster 4) and H21 (Cluster 5) were associated with specific morphological and chemical traits. Notably, Cluster 1 hybrids (H1, H12, H22, and H23) and control plants (ST1 and ST2) were positioned near the main trait contributors, reflecting their superior agro-morphological performance. In contrast, hybrids from other clusters (H2, H3, H5, and H11), despite contributing to the PCA dimensions, exhibited lower mean trait values. ST2 showed the highest contribution (7.9%), followed by H23 (7.6%), H11 (7.4%), and H12 (7.1%) (Figure 5). These hybrids are positioned closest to the main yield-related trait vectors in the PCA biplot (Figure 4b), confirming their superior agronomic performance and their strong influence on the overall multivariate structure. Hybrids H5, H3, H2, H15, H18, and ST1 also made substantial contributions, reflecting their distinct morphological or chemical profiles.

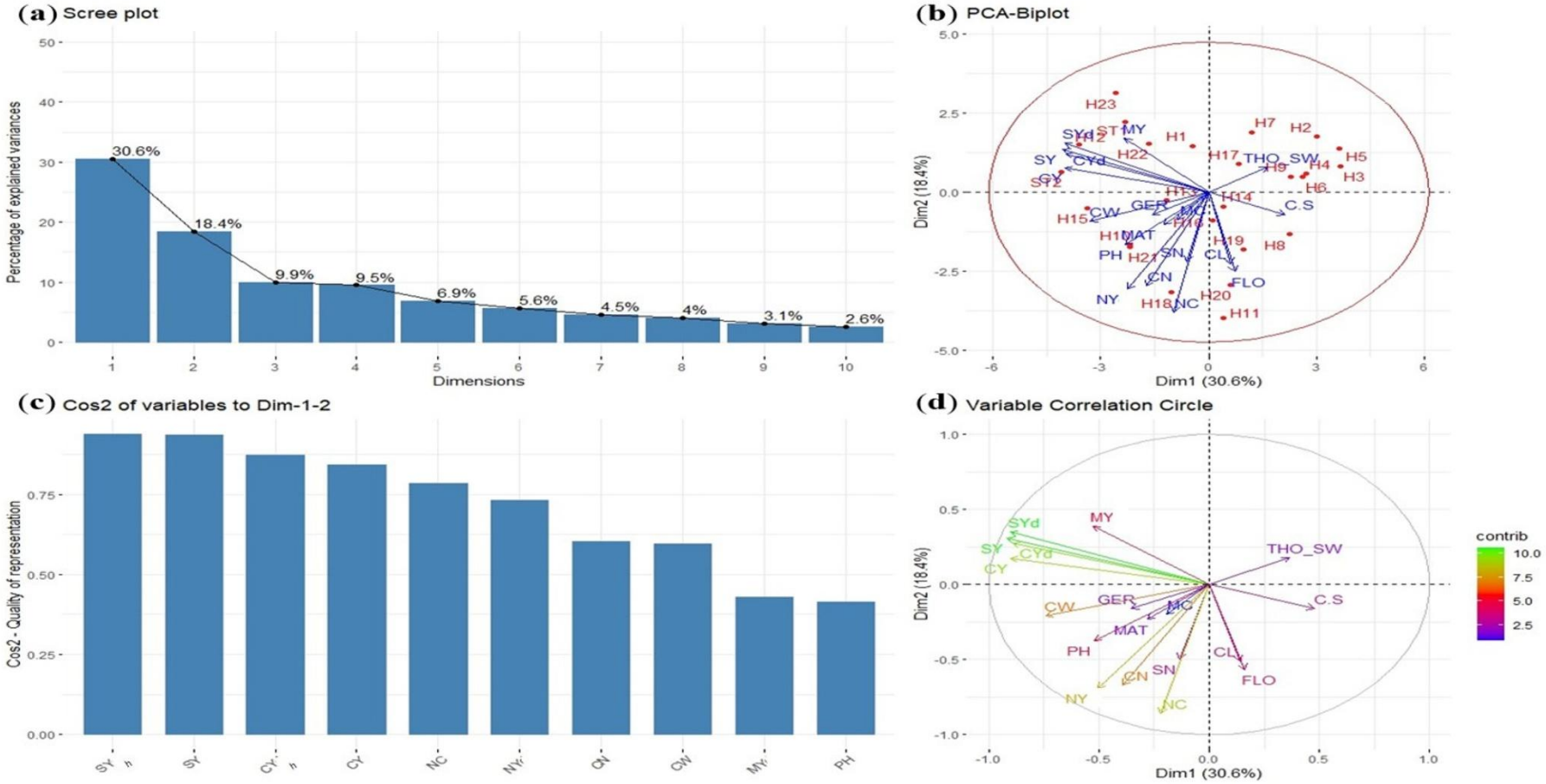

Figure 4. Scree plot of the dimensions (a), PCA-Biplot (b), Variable importance for Dim 1 and Dim 2 (c), and Variable correlation circle (d) (CN, Capsule Number; CL, Capsule Length; CW, Capsule Width; CY, Capsule Yield; C/S, Capsule / Seed; GER, Germination Time; FLO, Flowering Time; PH, Plant Height; SN, Stigma Number; SY, Seed Yield; MC, Morphine Content; NC, Noscapine Content; MAT, Maturing Time; MY, Morphine Yield per Hectare; NY, Noscapine Yield per Hectare; 1000-SW, 1000 Seed Weight).

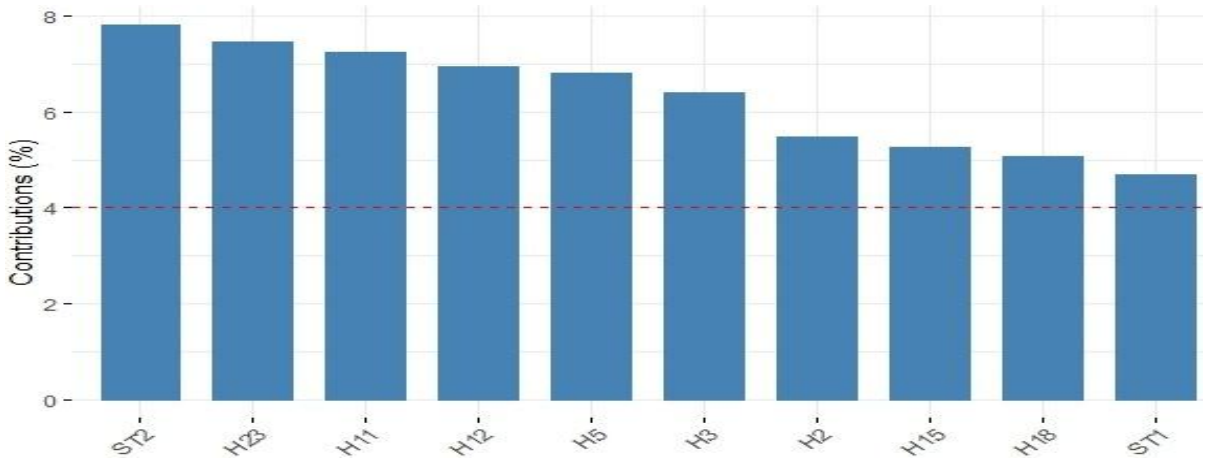

Figure 5. Contribution of top ten individual poppy hybrids to Dimension 1-2

Genetic Parameters for Selection

Estimates of genetic variability parameters are presented in Table 3. Because of the negative genotypic and phenotypic values of germination time, flowering time, and maturing time; they were excluded for genetic parameters estimations. Capsule and seed yield (kg ha^{-1}) exhibited the highest GV and PV, with high h^2 (0.86), and high GAM (55.38% and 54.64%), indicating strong additive genetic control and high potential for selection. They also displayed the highest GCV and PCV. Capsule number also showed high h^2 (0.73) and GAM (73.71%). In contrast, traits such as C/S ratio, stigma number, noscapine content and yield, and morphine yield displayed high ECV (> 30%) and low h^2, suggesting greater environmental influence and lower selection efficiency. Lower ECV (≤15.00) were determined for plant height (9.19), capsule length (10.58), capsule width (11.88), capsule and seed yield (kg ha^{-1}) (0.86 and 11.44).

Table 3. Genetic variability parameters of the opium poppy hybrids based on the studied traits

Traits	Grand Mean	Min	Max	GV	PV	GCV (%)	PCV (%)	ECV (%)	h^2	GA	GAM (%)
PH	98.25	63.99	130.46	122.96	204.46	11.28	14.55	9.19	0.60	17.72	18.03
CN	4.97	1.70	10.09	4.31	5.88	41.76	48.73	25.12	0.73	3.66	73.71
CL	50.67	36.71	71.72	4.15	32.89	4.02	11.32	10.58	0.12	1.48	2.94
CW	40.20	27.72	63.28	27.84	50.65	13.12	17.70	11.88	0.55	8.06	20.04
CY	3.22	1.17	5.85	0.78	1.27	27.40	35.08	21.90	0.61	1.42	44.10
SY	3.45	0.98	6.88	1.02	1.94	29.33	40.44	27.84	0.52	1.51	43.84
C/S	1.00	0.30	2.08	0.01	0.11	5.11	33.37	32.99	0.02	0.02	1.61
SN	10.84	5.80	102.58	0.98	32.77	9.15	52.83	52.03	0.03	0.35	3.26
CY_h	1086.0	57.20	175.40	980.61	1128.03	28.84	30.93	11.18	0.86	60.14	55.38
SY_h	1121.8	57.73	183.96	1027.08	1191.70	28.57	30.77	11.44	0.86	61.29	54.64
1000-SW	0.50	0.20	1.63	0.03	0.03	35.15	38.72	16.24	0.82	0.33	65.75
MC	0.93	0.34	2.07	0.04	0.11	22.49	36.38	28.59	0.38	0.27	28.67
NC	0.27	0.01	1.01	0.04	0.06	70.88	88.74	53.39	0.64	0.31	116.63
MY_h	10.5	0.44	2.94	0.11	0.22	30.94	44.85	32.47	0.48	0.46	43.97
NY_h	3.1	0.01	1.56	0.05	0.08	76.47	95.43	57.12	0.64	0.38	126.20

C/S: Capsule/seed, CL: Capsule length, CN: Capsule number, CW: Capsule width, CY: Capsule yield, ECV: Environmental coefficient of variation, FLO: Flowering time, GA: Genetic advance, GAM: Genetic Advance as percentage of mean, GCV: Genotypic coefficient of variation, GER: Germination time, GV: Genotypic variance, h^2: broad sense heritability, MAT: Maturing time, Max: Maximum, Min: Minimum, MC: Morphine content, MY_h: Morphine yield per hectare, NC: Noscapine content, NY_h: Noscapine yield per hectare, PCV: Phenotypic coefficient of variation, PH: Plant height, PV: Phenotypic variance, SN: Stigma number, 1000-SW: 1000 seed weight, SY: Seed yield.

4. DISCUSSION

Integrated Multivariate Analysis for Candidate Parental Selection

The agronomic performance means showed substantial GV, consistent with poppy germplasm (Lahiri et al., 2018; Singh et al., 2023) and among Turkish hybrids (Özgen et al., 2026) in previous studies. Germination (10.51-20.08 days) and maturation (244.14-276.83 days) were broadly comparable to the ranges reported for poppy grown under comparable Ankara conditions (Kahraman-Yanardağ et al., 2024). The highest-yielding material was a standard cultivar (ST2, 1491.90 kg ha^{-1}), which exceeded all hybrids, including the leading Cluster 1 hybrids (H15, 1414.00 kg ha^{-1}; H12, 1371.10 kg ha^{-1}), paralleling the report of Kahraman-Yanardağ et al. (2024), in which a registered cultivar achieved the highest yield in Ankara. Although the superior hybrids surpassed the lower-yielding standard (ST1, 1255.1 kg ha^{-1}), they did not exceed the best standard and are therefore best regarded as candidate parents valued for their divergent, heritable trait combinations rather than for immediate superiority.

The current study also demonstrated the value of integrating cluster analysis, PCA, and genetic parameter estimation for the efficient selection of superior opium poppy hybrids. While univariate analysis identified high-performing hybrids such as ST2, H12, and H15 for yield-related traits, multivariate analyses further revealed their genetic divergence and trait combinations. Cluster analysis distinguished high-yielding (Cluster 1), morphologically distinct (Cluster 4), and alkaloid-rich (Cluster 5) hybrids, thereby providing a practical framework for selecting genetically diverse candidate parents, consistent with previous reports emphasizing the importance of multivariate approaches in breeding programs (Jankulovska et al., 2013; Nematolahi et al., 2024). Similarly, PCA identified yield-related traits as the major contributors to overall variation, while hybrids such as H18 and H21, despite their relatively lower yield performance, were recognized as valuable genetic resources because of their unique morphological and alkaloid profiles. These findings demonstrate that integrating multivariate analyses with conventional performance evaluation enables more comprehensive candidate parental selection and helps overcome the limitations of selection based solely on individual traits (Lahiri et al., 2018; Singh et al., 2023).

The effectiveness of this integrated selection strategy is further supported by previous studies on Turkish opium poppy germplasm, which have consistently demonstrated extensive genetic variability for agronomic performance, alkaloid composition, and combining ability. Gümüşçü (2002), Doğramacı (2013), and Valizadeh et al. (2015) reported significant heterosis, heterobeltiosis, and both general and specific combining abilities for yield- and alkaloid-related traits, highlighting the importance of exploiting genetically diverse parents in hybrid breeding. Likewise, molecular and phenotypic characterization of Turkish germplasm revealed substantial variation in morphine content and key agronomic traits, while association mapping identified markers linked to morphine content, plant height, capsule index, and yield-related characteristics (Çelik et al., 2016). Collectively, these findings corroborate the current results by demonstrating that the integration of phenotypic performance, multivariate statistical analyses, and genetic diversity information provides a robust framework for identifying superior parental combinations and accelerating the development of high-yielding, alkaloid-rich opium poppy cultivars.

Environmental Modulation and Hybrid Stability

Significant year effects, particularly the reduced performance in 2023, highlight the sensitivity of opium poppy hybrids to environmental variation (Table 1). At the trait level, capsule number and seed yield declined in 2023, reflecting fewer capsules and reduced seed set, while germination duration lengthened from 10.51 to 20.08 days and maturation extended from 244.14 to 276.83 days, indicating a slowed, prolonged growth cycle. The consistently higher PV than GV confirmed the substantial environmental contribution to trait expression. This influence was most pronounced for alkaloid traits: a high ECV for noscapine yield, together with significant year × hybrid interactions for noscapine content, indicates that alkaloid accumulation is strongly environment-dependent, reducing selection efficiency and complicating the development of stable alkaloid profiles. These findings are consistent with Özgen (2026), who demonstrated that seasonal climatic variation markedly altered both capsule yield and benzylisoquinoline alkaloid composition in Turkish poppy chemotypes. Similarly, Bulut (2015) identified temperature, precipitation, relative humidity, and sunshine duration as the principal climatic determinants of poppy productivity, while Arslan et al. (2016) reported that the morphine content of the cultivar TMO-T varied from 0.87% to 1.11% and total alkaloid content from 2.96% to 3.40% when grown under the contrasting environmental conditions of Ankara and Tokat. In contrast, the relatively low ECV and consistent performance of Cluster 1 hybrids across years indicate greater stability and environmental adaptability for yield-related traits. Therefore, future breeding programs should combine the selection of high-yielding, environmentally stable hybrids with multi-environment evaluation to identify cultivars capable of maintaining consistent alkaloid profiles under diverse climatic conditions.

Genetic Architecture and Implications for Breeding Strategy

Genetic parameter estimates provide valuable insights into the inheritance of complex traits and the design of effective breeding strategies. In the current study, plant height, capsule yield, and seed yield exhibited the highest GV and PV,

indicating substantial exploitable genetic variation. The high GCV and PCV coefficients, together with high broad-sense h^2 and GA for capsule and seed yields, suggest that these traits are predominantly controlled by additive gene action and can be effectively improved through direct phenotypic selection (Singh et al., 2004; Tiwari et al., 2008; Mohammadi et al., 2023; Tena et al., 2023). Likewise, high h^2 estimates ($h^2 \geq 0.60$) for plant height, capsule number, and thousand-seed weight indicate strong genetic control and relatively low environmental interference (Mishra et al., 2016; Sanchez et al., 2023). However, h^2 should be interpreted together with GA, because high h^2 alone does not necessarily reflect breeding value (Roka et al., 2024). Accordingly, the high h^2-GA combination observed for capsule and seed yields confirms their suitability for selection in early generations, whereas the relatively low GA for plant height despite high h^2 suggests a greater contribution of non-additive gene effects, making this trait more suitable for hybrid breeding (Adhikari et al., 2018).

In contrast, alkaloid-related traits were more susceptible to environmental factors. The relatively high environmental coefficient of variation (ECV), particularly for noscapine yield, indicates that environmental conditions substantially affect alkaloid accumulation despite moderate to high h^2, thereby reducing selection efficiency under variable environments. This interpretation is supported by previous studies demonstrating extensive genetic diversity and strong genotype × environment interactions in Turkish opium poppy germplasm. Kara and Baydar (2021) reported significant variations in capsule yield and major alkaloids among registered cultivars, whereas Çelik et al. (2016) identified molecular markers associated with morphine content and key agronomic traits, highlighting the value of marker-assisted selection. Likewise, Özgen et al. (2024) and Yurdagül et al. (2024) showed that morpho-phenological, agronomic, and alkaloid traits effectively discriminate genetically distinct hybrids using multivariate approaches. Taken together, these findings indicate that combining genetic parameter estimation with phenotypic, multivariate, and molecular analyses provides a robust framework for identifying superior parental lines and accelerating the development of high-yielding, alkaloid-rich, and environmentally adapted opium poppy cultivars.

Proposed Crossing Strategy for Transgressive Improvement

Based on the trait divergence identified among clusters, we propose a strategic crossing scheme to maximize genetic gain. Cluster 1 hybrids (H12, H15, and H23) were proposed as yield contributors because their capsule and seed yields corresponded with high h^2 and GA, indicating additive control for early generation selection. H18 (Cluster 4) contributed a distinct morphology (high stigma and capsule width), and H21 (Cluster 5) contributed plant height and elevated alkaloid content. In opium poppy, opium yield is frequently inversely related to morphine content (Yadav et al., 2006; Maurya et al., 2019), so combining high yield with elevated alkaloid content is a recognized breeding challenge. Consistent with this, high-yielding and high-noscapine hybrids segregated into separate clusters in the current study; crossing yield contributors with H21 is therefore intended to recombine these divergent trait complexes.

These should first be crossed as H12/H15/H23 × H18 and × H21 to generate F_1 populations. Following F_2 selection for yield, the best recombinants from each cross can be intercrossed to pyramid advantageous alleles. To manage this potential trade-off, selection in segregating generations should be index-based rather than trait-by-trait, retaining recombinants with acceptable alkaloid contents without compromising yield (Maurya et al., 2019). Because alkaloid traits showed high environmental variance, multi-environment testing is required to distinguish stable from environmentally driven expression.

Elevated h^2 of yield-related traits indicates that effective selection can be initiated in early breeding generations, whereas the combination of moderate h^2 and GA for alkaloid-related traits suggests that greater selection efficiency may be achieved in later generations after population stabilization. This approach leverages both additive genetic variance and genetic distance to maximize transgressive segregation; however, empirical validation in future crossing studies is required. Accordingly, because combining-ability data are not yet available, the proposed hybrids in the current study should be regarded as candidate parents rather than confirmed complementary combinations. Future studies should estimate general and specific combining ability through diallel or line × tester designs, quantify mid- and better-parent heterosis in the resulting F_1 hybrids, and validate transgressive segregation for yield and alkaloid traits in F_2-F_3 populations under multi-environment trials (Gümüşçü, 2002; Doğramacı, 2013; Valizadeh et al., 2017; Yadav et al., 2009).

5. CONCLUSION

The current study evaluated the agro-morphological and phytochemical diversity of 23 poppy hybrids and 2 standard cultivars using integrated univariate, multivariate, and genetic parameter analyses. Significant genetic variation was observed in key traits, particularly plant height, capsule and seed yields, and alkaloid content. Cluster analysis resolved the material into five groups, and PCA identified seed and capsule yields as the main drivers of variation. Genetic parameter estimates showed that capsule and seed yields are governed largely by additive gene action, with high h^2 coupled with high GA, supporting direct selection in early generations. Parental candidates were selected based on explicit criteria: high heritable yield potential (H12, H15, and H23; Cluster 1), morphological distinctness (H18; Cluster 4), and elevated alkaloid content (H21, Cluster 5). Because these hybrids were distinguished by genetic divergence rather than

by combining ability data, they should be regarded as candidate parents whose potential to generate transgressive segregation requires empirical validation in subsequent crossing studies. Overall, integrating genetic parameters with multivariate analysis provides a practical framework for parental selection in *Papaver somniferum* breeding.

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