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CHARACTERIZATION OF CAPSULE MORPHOLOGIC PROPERTIES AND ALKALOID CONTENTS OF TURKISH POPPY GENOTYPES WITHIN ANKARA ENVIRONMENTAL CONDITIONS

Y. K. Yanardağ, S. Day*and N. Bayraktar

Ankara University, Faculty of Agriculture, Department of Field Crops, 06110 – Dışkapı – Ankara – Türkiye
*Corresponding Author: Sibel Day. Email: day@ankara.edu.tr

ABSTRACT

Poppy (*Papaver somniferum*) L. is cultivated commercially in several countries legally to produce alkaloids (i.e. morphine, codeine, thebaine, narcotine, papaverine and oripavine) for pharmaceutical industries. The demand for these alkaloids is increasing and Global climate change is influencing poppy cultivated areas. Türkiye is responsible for poppy cultivation, breeding new cultivars and adaptation trials. Therefore, the present investigation was undertaken during two years to examine the most cultivated 16 cultivars and 3 new lines for capsule morphology properties, their alkaloid values and capsule yield in Ankara conditions which is not in the poppy cultivation area in Türkiye. The experiment was arranged in the randomized complete block design with four replications. The plot size of 2.4 m² (2 m × 1.2 m) and 4 rows with spacing 30 cm between rows and 10 cm within plants were applied. The capsule parameters and alkaloid content were measured in 10 dry capsules after harvesting. To measure the content of alkaloids (%) the capsules were ground into powder and HPLC-MS/MS analysis after the solvent extraction of poppy straw on dry matter was conducted. Genotypes showed variation in stigmatic rays and the number of stigmatic rays was higher in the first year. The maximum stigmatic ray value was seen in cv. Ofis-95 with 13.27 in the first year. The maximum value for capsule length and diameter was obtained from cv. Ofis-NP (51.75 mm) and cv. Ofis-95 (45.00 mm) in the mean of two years. The maximum capsule yield was obtained from cv. Ofis-95 for both years and the values were 155.90 and 160.30 kg da^{-1}. The maximum morphine percentage was recorded in cv. Ofis-1 with 1.63 and 1.24% respectively. The percentage of noscapine, codeine, thebaine was noted as maximum values in Ofis-1 among genotypes. The results favoured the cultivation of these genotypes in Ankara which is not one of the poppy cultivation area and further agronomic studies should be undertaken to develop production technology of poppy in these environmental conditions.

Keywords: *Papaver somniferum,* cultivation, capsule yield, morphine, noscapine

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INTRODUCTION

Türkiye is among the main eight countries (Türkiye, India, Spain, Hungary, Czechia, France, Australia and China) of the world, that are recognised to grow poppy legally in accordance with UN adopted narcotic protocol of 1948 and UN Convention on Narcotics, 1988. As well as monitoring agricultural techniques and controlling fields, the treaty requires participating nations to develop a novel approach to the creation of new cultivars.

The alkaloid spectrum of the Papaver L. genus has been analysed, and around 140 alkaloids have been isolated. These alkaloids are part of the isoquinoline family and biogenetically derived variants. *Papaver somniferum* L. is known to contain the highest amount of morphine, usually ranging from 45% to 90% of the alkaloid content, although the content of other alkaloids is generally low (Yazici and Yilmaz, 2021).

Poppy farming is a significant industry in Türkiye, particularly for the production of seeds and capsules. The global growing demand for alkaloids has resulted in need to increase legal poppy cultivation, which in turn could create a balance between demand and supply. The productivity is influenced by weather conditions, especially temperature and rainfall during germination, flowering, and capsule drying stages (Lal *et al*., 2014; Yazici, 2022). Management of soil cultivation practices for poppy in the fast-warming climatic conditions and stretching them to other areas with appropriate climatic conditions as recommended by Turkish Grain Board, could help to control several abiotic and biotic stresses and to improve current yield level of the crop. The opium poppy plant has over 40 different alkaloids. Among them, morphine is the most prominent and is known for its powerful natural pain-relieving properties. Codeine and noscapine are used to suppress coughs while papaverine is a relaxant for smooth

muscles. Thebaine, on the other hand, isn't directly used for therapeutic purposes. However, it is transformed into other pain relievers in the industrial sector (Sousa *et al.,* 2022).

Breeding new poppy cultivars with high morphine yield has been studied to meet the technological production needs for opium. Opium is derived from the dried capsules of the poppy plant instead of traditional opium production methods. Consequently, certain promising cultivars have been enhanced and disseminated to farmers. However, the breeding of new varieties with higher morphine yields and their swift distribution to farmers remains insufficient. In addition, new poppy cultivating areas should be searched out under the changing climate conditions scenario. The Global warming is also influencing the poppy cultivation fields in Türkiye and searching new areas for poppy production is important. Poppy plants come across with stress factors threatening the crop. Evaluating the genotypes cultivated in Türkiye for their capsule morphology and alkaloids in Ankara conditions which is not one of the poppy cultivation areas is important. The cultivars' adaptation capacity, capsule yield, and alkaloid yield should be assessed for other regions.

In response to above-mentioned discussion, this study aimed to evaluate the diversity in capsule morphology, capsule yield, and alkaloid composition among 19 selected poppy genotypes within the hot and humid dSA type continental climate of Ankara. It's worth noting that Ankara is not officially designated as a specific poppy cultivation area in the official gazette of Türkiye.

MATERIALS AND METHODS

Nineteen genotypes of *Papaver somniferum* seeds were utilized in this experiment. The poppy genotypes were cultivated during 2019-2020 and 2020-2021 seasons at the experimental field of the Department of Field Crops, Faculty of Agriculture, and Ankara University. The field situated at an altitude of 860 meters and it lies between 30° 53' north latitude and 32° 45' west longitude. The genotypes were obtained from Turkish Grain Board (Ofis-1, Ofis-2, Ofis-3, Ofis-4, Ofis-8, Ofis-NP, Ofis-NM, Ofis-95, Ofis-96, TMO-1, TMO-2, TMO-3, Afyon-95), Republic of Türkiye Ministry of Agriculture and Forestry Transitional Zone Agricultural Research Institute (Hüseyinbey, Seyitgazi, Çelikoğlu) and Ankara University Faculty of Agriculture, Department of Field Crops (Line-8, Line 15, Line 21).

The study was structured as a randomized complete block design with four replications. Sowing occurred on October 26, 2019, and October 27, 2020. Harvesting took place on July 27, 2020, and July 12, 2021, for the respective years. The experiment was conducted with a plot size of 2.4 m² (2 m x 1.2 m) and 4 rows with spacing 30 cm between rows and 10 cm within plants. Standard cultural practices were followed throughout crop season. To maintain 4 kg da^{-1} N, diammonium phosphate was applied before sowing. Soon after sowing, irrigation for seedling emergence was carried out and no further irrigation was done afterwards. The 1st manual weeding was done in March while thinning, second manual weeding and earthing up took place in April.

The capsules were hand-harvested when fully ripe, considering the maturity of the cultivars. The capsule yield (kg da^{-1}), capsule number per plant, capsule length (mm), and capsule width (mm) were measured as described by Kara (2017). The capsules were left to dry at room temperature in the shade until their moisture content reached 10±0.10%. Subsequently, the seeds were separated from the capsules, and the capsule yield (kg da^{-1}) was calculated by multiplying the capsule weight by 1000 and dividing it by the plot size (m^2).

Phytochemical analysis: Ten capsules were randomly selected from each plot for alkaloid analysis of genotypes. The capsules were ground into powder and placed in 5 g bags. The laboratory at Afyon Alkaloids Factory determined the content (%) of morphine, codeine, oripavine, thebaine, papaverine, and noscapine through HPLC-MS/MS analysis after solvent extraction of poppy straw on dry matter using an HPLC device (Yazici, 2022).

To prepare the alkaloid analysis sample a 0.5 g capsule was heated with 1.5 ml of water. After 15 minutes 2 g of aluminium oxide was added and mixed. The solution was then dissolved in 4.1 g of sodium acetate and 25 ml of glacial acetic acid was added. The pH of the mixture was reduced to 3.6, and water was added to make it up to 1000 ml. The solution was filtered through a Millipore, 880 ml of the mixture was taken, and 80 ml of acetonitrile and 40 ml of absolute alcohol were added to make it up to 1000 ml. The solution was de-aerated in an ultrasonic bath for 30 minutes (Kara, 2017).

The conditions of HPLC were: column, 300 mm × 3.9 mm Bundapak C18; flow, 1.5 ml min^{-1}; standard, 0.1 mg ml^{-1} (Barberi-Heyob *et al.,* 1991).

To fill the column of the device, 25 ml of water was added, and 8 g of aluminium oxide was added to the column. The mixture was allowed to accumulate at the bottom, and water was taken. Then 5 ml of water was added. The analysis sample was placed in the column and filled with pure water to ensure that there is 50 ml in the volumetric flask.

The device was allowed to flow at 1.5 ml $minute^{-1}$ and the buffer solution was allowed to reach equilibrium. The standard was found by adding 10 microliters of 0.1 mg l^{-1} to the solution. To complete the solution to 50 ml, the sample was filtered through a

millipore and ten microliters were added (Karadavut and Arslan, 2006).

Soil analysis: The physical and chemical characteristics of the soil where the experiment was carried out were analysed at the laboratory of the Central Forestry Soil, Fertilizer, and Water Resources Research Institute in Ankara, Türkiye. Soil samples were collected from 0 to 30 cm deep (Day *et al.,* 2023).

Statistical analysis: The experimental design was randomised complete block design. Data for morphine, noscapine, codeine, thebaine, papaverine, and oripavine were subjected to arcsine transformation before ANOVA. MSTAT-C 2.1 software program (Michigan State University, 1991) was used for all statistical analysis. The differences between the means were compared using Duncan's Multiple Range Test.

RESULTS

Soil and climate properties: The experimental site had a soil texture of clay and clay loam (Table 1). The soil had a slightly alkaline nature and contained low levels of nitrogen and organic matter. In 2019-2020, the percentage of $CaCO_3$ was 5.15%, which increased to 7.27% in 2020-2021. The available P_2O_5 content was found to be 3.04 kg da^{-1} and 5.53 kg da^{-1} in the respective years. Available potassium (K_2O) concentration was measured as 117.82 kg da^{-1} and 128.08 kg da^{-1} in the respective years.

Table 1. Soil analysis report of the experimental site.

Soil test report (0-30 cm in depth)	1. year	2. year
Organic matter (%)	0.61	0.45
Lime - CaCO3 (%)	5.15	7.27
Available phosphorus - P_2O_5 (kg da^{-1})	3.04	5.53
Available potassium - K_2O (kg da^{-1})	117.82	128.10
pH	7.88	8.08
Total nitrogen - N (%)	0.093	0.059
Salinity (%)	0.0679	0.0347
Soil texture	Clay	Clay-loam
Electrical conductivity (dS m^{-1})	1.33	0.81

Soil Quality and Productivity Analysis Laboratory of the Ministry of Agriculture and Forestry, Ankara, Türkiye

The long term average humidity of the experimental site is 60.40%, with a long-term average temperature of 11.90 °C and an annual rainfall average of 395.10 mm, according to the General Directorate of Meteorology in 2022. Table 2 provides the temperature, relative humidity, and precipitation during the growing season, as well as the long-term average of meteorological data.

Table 2. Meteorological data of the experimental site

Months	Precipitation (mm)			Temperature (°C)			Relative Humidity (%)		
	2019-2020	2020-2021	Average 1929-2022	2019-2020	2020-2021	Average 1929-2022	2019-2020	2020-2021	Average 1929-2022
10	7.50	29.60	27.60	16.40	17.70	13.20	48.20	45.30	58.50
11	21.00	5.90	31.70	10.30	6.80	7.20	58.30	60.20	69.80
12	66.20	17.70	44.60	4.20	6.00	2.50	80.30	71.10	77.60
1	22.40	65.80	41.20	1.40	3.50	0.20	72.80	74.10	77.30
2	70.40	16.80	35.70	4.20	4.50	1.90	66.20	60.50	72.70
3	23.20	69.50	40.00	9.30	5.10	5.80	56.40	62.70	64.40
4	23.40	29.30	43.00	11.70	12.30	11.20	47.40	54.90	58.60
5	69.40	13.70	51.30	17.00	19.10	16.00	48.90	39.70	57.00
6	108.70	41.20	35.90	20.70	19.00	20.00	50.90	57.40	52.10
7	5.10	1.90	14.20	25.90	25.50	23.40	37.40	37.20	44.40

Directorate General of Meteorology, Ankara, Türkiye 2022.

Capsule morphologic properties: The capsule number per plant did not vary among genotypes and the number of capsules were between 2.05 and 4.25 (Table 3). Years × genotypes showed statistically significant interaction on number of stigmatic rays between years ($p \leq 0.01$). Furthermore, significant differences ($p \leq 0.01$) among

genotypes and between years ($p \leq 0.01$) were also noted. Evaluation of interaction showed that the maximum and minimum number of stigmatic rays were observed in Ofis-8 in 2020 and TMO-1 in 2021 with 13.70 and 8.25, respectively (Table 3).

Table 3. The comparison of means in capsule number and number of stigmatic rays of genotypes

Genotypes	Capsule number per plant			Number of stigmatic rays		
	2019-2020	2020-2021	Average	2019-2020	2020-2021	Average
Ofis-1	3.05	4.25	3.65	13.10±0.38^{ab}	12.65±0.14^{a-d} **	12.88±0.24^{a} **
Ofis-2	2.05	3.18	2.61	12.27±0.11^{a-f}	9.75±0.48^{i-n}	11.00±0.52^{b-g}
Ofis-3	3.28	3.70	3.49	13.65±0.16^{a}	10.25±0.48^{g-n}	11.95±0.68^{abc}
Ofis-4	3.30	2.45	2.88	10.75±0.63^{d-l}	9.87±0.42^{i-n}	10.30±0.39^{d-g}
Ofis-8	3.73	4.08	3.90	13.70±0.16^{a}	8.75±0.48^{lmn}	11.21±0.96^{b-f}
OFİS-NP	3.43	3.90	3.66	11.75±0.36^{a-i}	9.52±0.51^{j-n}	10.64±0.51^{c-g}
OFİS-NM	3.13	3.60	3.36	12.02±0.31^{a-g}	10.75±0.8^{d-i}	11.38±0.47^{b-e}
Ofis-95	3.93	3.33	3.63	13.27±0.21^{ab}	11.00±0.91^{c-k}	12.13±0.61^{ab}
Ofis-96	3.13	2.70	2.91	12.85±0.12^{abc}	10.00±0.41^{h-n}	11.43±0.57^{bcd}
TMO-1	3.45	3.25	3.35	12.00±0.41^{a-h}	8.25±0.19^{n}	10.13±0.74^{d-g}
TMO-2	2.30	3.18	2.74	11.75±0.25^{a-i}	10.43±0.38^{f-m}	11.08±±0.33^{b-f}
TMO-3	2.00	3.28	2.64	11.50±0.65^{b-j}	8.50±0.29^{mn}	10.00±0.66^{efg}
Afyon-95	3.38	4.03	3.70	12.80±0.44^{abc}	10.25±0.48^{g-n}	11.53±0.57^{bcd}
Hüseyinbey	3.03	3.23	3.13	12.35±0.23^{a-f}	9.25±0.51^{k-n}	10.80±0.64^{b-g}
Seyitgazi	2.88	3.55	3.21	12.50±0.29^{a-e}	10.50±0.29^{e-m}	11.50±0.42^{bcd}
Çelikoğlu	3.58	3.08	3.33	11.75±0.48^{a-i}	9.87±0.39^{l-n}	10.81±0.46^{b-g}
Line-8	2.58	3.35	2.96	10.57±0.42^{e-l}	8.75±0.56^{lmn}	9.67±0.47^{g}
Line-15	3.70	3.05	3.38	10.50±0.64^{e-m}	9.32±0.67^{k-n}	9.90±0.48^{fg}
Line-21	3.58	3.68	3.63	12.25±0.3^{a-g}	10.25±0.63^{g-n}	11.23±0.50^{b-f}
Average	3.13	3.41		12.18±0.13 A	9.89±0.15 B **	

**Means followed by the same letter(s) are not different ($p \leq 0.01$)

There were significant differences ($p \leq 0.01$) among the capsule length values of genotypes for the average of two years (Table 4). The maximum capsule length was recorded in Çelikoğlu cultivar (with 52.00 mm) while the minimum value was noted in Ofis-96 (with 43.62 mm).

There were significant differences ($p \leq 0.01$) in the capsule diameter of poppy among different genotypes over an average of two years. Ofis-95 had the maximum capsule diameter of 45.00 mm, while Ofis-4 had the minimum capsule diameter of 34.50 mm. Significant differences in capsule diameter between years ($p \leq 0.01$) were also found by analysis of variance.

Table 4. Capsule length and capsule diameter means of genotypes

Genotypes	Capsule length (mm)			Capsule diameter (mm)		
	2019-2020	2020-2021	Average	2019-2020	2020-2021	Average
Ofis-1	49.50	49.50	49.50±1.18^{ab} **	45.00	41.00	43.00±0.92^{ab} **
Ofis-2	49.50	49.00	49.25±0.90^{ab}	39.00	35.00	36.87±1.16^{def}
Ofis-3	50.00	49.75	49.87±0.97^{ab}	35.00	38.00	36.37±0.96^{ef}
Ofis-4	49.50	46.50	48.00±1.08^{ab}	37.00	32.00	34.50±1.07^{f}
Ofis-8	48.25	48.50	48.37±1.08^{ab}	41.00	38.00	39.62±1.15^{b-e}
OFİS-NP	52.00	51.50	51.75±0.75^{a}	39.00	36.00	37.50±1.60^{c-f}
OFİS-NM	49.00	50.25	49.62±0.98^{ab}	45.00	44.00	44.25±1.30^{a}
Ofis-95	53.25	50.50	51.87±1.01^{a}	46.00	43.00	45.00±0.99^{a}
Ofis-96	43.25	44.00	43.62±0.70^{c}	44.00	38.00	41.00±1.24^{a-d}
TMO-1	48.00	48.25	48.12±0.89^{ab}	39.00	37.00	38.12±0.99^{c-f}
TMO-2	50.00	47.75	48.87±1.12^{ab}	44.00	40.00	41.75±1.46^{abc}
TMO-3	50.00	49.25	49.62±0.84^{ab}	36.00	34.00	35.00±1.05^{f}
Afyon-95	46.75	48.00	47.37±0.78^{b}	40.00	37.00	38.50±1.50^{c-f}
Hüseyinbey	49.50	48.75	49.12±1.21^{ab}	39.00	36.00	37.50±1.53^{c-f}
Seyitgazi	49.25	50.00	49.62±1.65^{ab}	43.00	38.00	40.37±1.27^{a-e}
Çelikoğlu	51.50	52.50	52.00±0.57^{a}	37.00	35.00	36.00±1.19^{ef}
Line-8	49.00	51.25	50.12±1.35^{ab}	42.00	39.00	40.50±1.21^{a-e}
Line-15	48.75	46.25	47.50±0.82^{b}	39.00	35.00	37.00±0.89^{def}
Line-21	49.75	51.50	50.62±0.92^{ab}	40.00	36.00	38.00±1.06^{c-f}
Average	49.30	49.11		40.53±0.43 A	37.40±0.48 B **	

** Means followed by the same letter(s) are not different ($p \leq 0.01$)

Statistically significant differences identified in terms of capsule dry weight per plant ($p \leq 0.01$) among genotypes. The highest capsule dry weight determined in Ofis-95 cultivar with 4.51 g plant^{-1} while the lowest capsule dry weight determined in TMO-3 with 2.79 g plant^{-1} (Table 5).

Years × Genotypes showed statistically significant interaction ($p \leq 0.01$) on capsule yield. The evaluation of interaction reflected that Ofis-95 gave the highest value for capsule yield both harvest years with 155.90 g da^{-1} and 160.30 g da^{-1}. The lowest value for capsule yield was observed in cv. TMO-3 with 89.30 g da^{-1} (Table 5).

In the average of the years there was a significant variation among genotypes ($p \leq 0.01$). Ofis-95 had the highest capsule yield with 158.10 g da^{-1}. The lowest capsule yield was obtained from TMO-3 with 97.60 g da^{-1} in Ankara conditions (Table 5).

Phytochemical properties: The composition of the alkaloids revealed that morphine was the major alkaloid in genotypes. Significant differences ($p \leq 0.05$) were observed in morphine contents for years × genotypes interaction. The highest morphine content was observed in 2020 harvest from Ofis-1 with 1.63 %. Ofis-1 gave the highest morphine content also among all genotypes in 2021 harvest. The minimum morphine content was obtained from Line-21 with 0.32% from 2020 harvest (Table 6). Noscapine showed significant differences in-between two years ($p \leq 0.01$). Noscapine contents were 0.123%-0.153% in 2020 and 2021 harvests respectively. It was also observed that noscapine showed a statistically important diversity among genotypes ($p \leq 0.01$). The maximum noscapine was determined in Ofis-1 with 0.446% and the minimum content was observed in TMO-3, Çelikoğlu and Line 8 with 0.073% (Table 6).

There were significant differences ($p \leq 0.01$) among codeine content of genotypes for the average of two years (Table 7). The maximum codeine content value was observed in Ofis-1 (0.204%) while the minimum was obtained from TMO-3 (0.075%).

Genotypes ($p \leq 0.01$) and years ($p \leq 0.01$) statistically influenced the thebaine content of poppy capsules. The maximum thebaine content among genotypes were noted in Ofis-NM (0.193%) and the minimum was noted in Ofis-95 (0.008%). Ofis-1 followed the Ofis-NM and according to Duncan Multiple Range test they were both in the same group. Thebaine content of 2020 harvest was less than 2021 harvest. The values were 0.038% and 0.048% respectively (Table 7).

Genotypes showed statistically importance ($p \leq 0.01$) in terms of papaverine content in poppy capsules. The highest value was observed in Ofis-8 with 0.099% while the lowest value was in TMO-3. The statistical differences among years were also found important ($p \leq 0.01$) and the value for 2020 and 2021 harvests 0.034 and 0.037 % respectively (Table 8).

Table 5. The comparison of means in capsule dry weight and capsule yield of genotypes

	Capsule dry weight per plant (g plant^{-1})			Capsule yield (kg da^{-1})		
	2019-2020	**2020-2021**	**Average**	**2019-2020**	**2020-2021**	**Average**
Ofis-1	3.00	3.40	3.20±0.19^{bcd} **	105.10±10.30^{bcd}	119.10±7.95^{bc}**	112.10±6.58^{cd} **
Ofis-2	3.60	3.75	3.68±0.23^{a-d}	126.10±13.40^{a-d}	131.40±11.30^{abc}	128.70±8.17^{a-d}
Ofis-3	3.60	3.75	3.68±0.17^{a-d}	126.10±6.39^{a-d}	131.40±11.30^{abc}	128.70±6.10^{a-d}
Ofis-4	3.15	4.00	3.58±0.21^{bcd}	110.30±1.75^{bcd}	140.10±10.10^{abc}	125.20±7.36^{bcd}
Ofis-8	2.85	3.18	3.01±0.24^{cd}	99.80±11.91^{cd}	111.20±13.43^{c}	105.50±8.58^{cd}
OFİS-NP	2.95	3.53	3.24±0.16^{bcd}	103.30±6.62^{bcd}	123.50±6.45^{bc}	113.40±5.72^{cd}
OFİS-NM	3.00	3.88	3.44±0.31^{bcd}	105.10±17.84^{bcd}	135.70±8.38^{abc}	120.40±10.81^{bcd}
Ofis-95	4.45	4.58	4.51±0.39^{a}	155.90±22.41^{a}	160.30±19.79^{a}	158.10±13.86^{a}
Ofis-96	3.75	3.90	3.83±0.25^{abc}	131.40±12.58^{abc}	136.60±13.63^{abc}	134.00±8.64^{abc}
TMO-1	3.50	3.75	3.63±0.30^{a-d}	122.60±16.54^{a-d}	131.40±15.15^{abc}	127.00±10.51^{a-d}
TMO-2	3.05	3.88	3.46±0.41^{bcd}	106.80±23.28^{bcd}	135.70±16.50^{abc}	121.30±14.29^{bcd}
TMO-3	2.55	3.03	2.79±0.28^{d}	89.30±16.02^{d}	106.00±11.73^{c}	97.60±9.72^{d}
Afyon-95	3.20	3.85	3.53±0.25^{bcd}	112.10±15.91^{bcd}	134.90±4.16^{abc}	123.50±8.74^{bcd}
Hüseyinbey	3.50	4.18	3.84±0.28^{abc}	122.60±13.25^{a-d}	146.20±13.81^{ab}	134.40±9.92^{abc}
Seyitgazi	2.70	3.78	3.24±0.25^{bcd}	94.60±6.06^{cd}	132.20±9.62^{abc}	113.40±8.85^{cd}
Çelikoğlu	4.05	4.20	4.13±0.26^{ab}	141.90±13.52^{ab}	147.10±3.90^{a}	144.50±7.38^{ab}
Line-8	3.35	4.08	3.71±0.23^{a-d}	117.03±10.45^{bcd}	142.70±7.35^{abc}	130.00±7.12^{a-d}
Line-15	3.25	3.63	3.44±0.12^{bcd}	113.80±5.98^{bcd}	127.00±0.00^{bc}	120.40±3.22^{cd}
Line-21	2.85	3.53	3.19±0.22^{bcd}	99.80±9.20^{cd}	123.50±10.63^{bc}	111.70±7.89^{cd}
Average	3.28	3.78		114.90	132.40	

** Means followed by the same letter(s) are not different ($p \leq 0.01$)

Table 6. Content of morphine and noscapine in a dried capsule based on dry weight.

Genotypes	Morphine (%)			Noscapine (%)		
	2019-2020	2020-2021	Average	2019-2020	2020-2021	Average
Ofis-1	1.63±0.34^{a}	1.24±0.27^{b} *	1.44±0.27^{a} **	0.376	0.516	0.446±0.43^{a} **
Ofis-2	0.77±0.29^{d-i}	0.99±0.15^{b-e}	0.88±0.20^{bcd}	0.076	0.190	0.133±0.22^{bc}
Ofis-3	0.91±0.28^{b-f}	0.89±0.24^{c-g}	0.90±0.17^{bc}	0.055	0.210	0.132±0.29^{bc}
Ofis-4	0.48±0.87^{j-m}	0.84±0.11^{d-h}	0.66±0.50^{def}	0.038	0.116	0.077±0.23^{c}
Ofis-8	0.59±1.00^{i-m}	0.83±0.05^{d-h}	0.71±0.51^{cde}	0.132	0.247	0.189±0.33^{bc}
OFİS-NP	0.76±0.07^{d-i}	0.73±0.28^{d-i}	0.75±0.13^{cde}	0.460	0.221	0.340±0.63^{ab}
OFİS-NM	1.22±0.43^{bc}	1.05±0.20^{bcd}	1.14±0.23^{ab}	0.158	0.175	0.167±0.27^{bc}
Ofis-95	0.54±0.23^{h-m}	0.61±0.16^{f-l}	0.58±0.14^{ef}	0.075	0.075	0.075±0.17^{c}
Ofis-96	0.51±0.18^{i-m}	0.60±0.16^{f-l}	0.55±0.13^{efg}	0.104	0.102	0.103±0.09^{c}
TMO-1	0.74±0.57^{e-i}	0.68±0.23^{e-j}	0.71±0.28^{cde}	0.077	0.110	0.093±0.16^{c}
TMO-2	0.77±0.34^{d-i}	0.70±0.14^{e-i}	0.73±0.17^{cde}	0.083	0.110	0.096±0.10^{c}
TMO-3	0.57±0.24^{h-l}	0.53±0.17^{i-m}	0.55±0.14^{efg}	0.055	0.092	0.073±0.17^{c}
Afyon-95	0.58±0.10^{g-l}	0.63±0.09^{f-k}	0.60±0.07^{def}	0.121	0.145	0.133±0.12^{bc}
Hüseyinbey	0.60±0.17^{f-l}	0.61±0.18^{f-l}	0.60±0.12^{def}	0.091	0.116	0.104±0.10^{c}
Seyitgazi	0.62±0.07^{f-l}	0.71±0.19^{e-i}	0.66±0.12^{cde}	0.133	0.115	0.124±0.15^{bc}
Çelikoğlu	0.71±0.28^{e-i}	0.64±0.12^{f-k}	0.68±0.15^{cde}	0.082	0.063	0.073±0.09^{c}
Line-8	0.54±0.42^{i-m}	0.48±0.08^{i-m}	0.51±0.20^{efg}	0.066	0.080	0.073±0.08^{c}
Line-15	0.37±0.05^{lm}	0.48±0.21^{i-m}	0.42±0.13^{fg}	0.084	0.108	0.096±0.16^{c}
Line-21	0.32 ±0.12^{m}	0.40±0.02^{klm}	0.36±0.09^{g}	0.074	0.116	0.095±0.12^{c}
Average	0.70	0.72		0.123±0.11 B	0.153±0.08 A **	

** Means followed by the same letter(s) are not different (p≤0.01)

Table 7. Content of codeine and thebaine in a dried capsule based on dry weight

Genotypes	Codeine (%)			Thebaine (%)		
	2019-2020	2020-2021	Average	2019-2020	2020-2021	Average
Ofis-1	0.218	0.190	0.204±0.22^{a} **	0.154	0.161	0.157±0.17^{a} **
Ofis-2	0.109	0.140	0.124±0.09^{b-e}	0.014	0.031	0.023±0.09^{bc}
Ofis-3	0.100	0.112	0.106±0.07^{b-e}	0.008	0.027	0.017±0.10^{bc}
Ofis-4	0.082	0.136	0.109±0.15^{b-e}	0.011	0.036	0.024±0.16^{bc}
Ofis-8	0.111	0.152	0.131±0.10^{bcd}	0.013	0.045	0.029±0.11^{bc}
OFİS-NP	0.113	0.090	0.101±0.26^{b-e}	0.011	0.015	0.013±0.09^{bc}
OFİS-NM	0.170	0.137	0.154±0.16^{ab}	0.199	0.186	0.193±0.43^{a}
Ofis-95	0.126	0.082	0.104±0.10^{b-e}	0.009	0.006	0.008±0.05^{c}
Ofis-96	0.117	0.075	0.096±0.11^{b-e}	0.013	0.034	0.023±0.15^{bc}
TMO-1	0.100	0.071	0.085±0.12^{de}	0.012	0.021	0.016±0.10^{bc}
TMO-2	0.096	0.072	0.084±0.11^{cde}	0.015	0.037	0.026±0.14^{bc}
TMO-3	0.092	0.060	0.075±0.12^{e}	0.011	0.039	0.025±0.15^{bc}
Afyon-95	0.112	0.077	0.094±0.11^{b-e}	0.012	0.018	0.015±0.09^{bc}
Hüseyinbey	0.118	0.104	0.111±0.09^{b-e}	0.047	0.034	0.040±0.16^{bc}
Seyitgazi	0.117	0.127	0.122±0.07^{b-e}	0.017	0.058	0.037±0.19^{bc}
Çelikoğlu	0.106	0.128	0.117±0.06^{b-e}	0.015	0.021	0.018±0.11^{bc}
Line-8	0.123	0.108	0.115±0.08^{b-e}	0.098	0.039	0.068±0.32^{b}
Line-15	0.080	0.086	0.083±0.08^{de}	0.014	0.032	0.023±0.12^{bc}
Line-21	0.150	0.131	0.140±0.06^{abc}	0.047	0.064	0.056±0.11^{bc}
Average	0.118	0.109		0.038±0.08 B	0.048±0.07A *	

** Means followed by the same letter(s) are not different (p≤0.01)

Analysis of variance showed statistically significant differences in oripavine content in poppy capsules between years (p≤0.01). The values were 0.015% for 2020 harvest and 0.020% for 2021 harvest. Furthermore, significantly different effects of genotypes were also noted in the oripavine content (in the range of

0.011-0.058%). The maximum value of oripavine was obtained in Ofis-NM (Table 8).

Table 8. Content of papaverine and oripavine in a dried capsule based on dry weight

Genotypes	Papaverine (%)			Oripavine (%)		
	2019-2020	2020-2021	Average	2019-2020	2020-2021	Average
Ofis-1	0.029	0.021	0.025±0.18$^{c\text{-}f\ **}$	0.019	0.029	0.024±0.11$^{b\ **}$
Ofis-2	0.074	0.044	0.059±0.16^{abc}	0.012	0.016	0.014±0.03^{b}
Ofis-3	0.003	0.016	0.010±0.08^{g}	0.011	0.012	0.012±0.02^{b}
Ofis-4	0.003	0.029	0.016±0.15^{fg}	0.013	0.018	0.015±0.03^{b}
Ofis-8	0.111	0.088	0.099±0.07^{a}	0.012	0.016	0.014±0.03^{b}
OFİS-NP	0.039	0.072	0.056±0.23$^{b\text{-}e}$	0.010	0.013	0.012±0.02^{b}
OFİS-NM	0.053	0.034	0.044±0.14$^{b\text{-}f}$	0.051	0.065	0.058±0.16^{a}
Ofis-95	0.009	0.025	0.017±0.12^{efg}	0.013	0.022	0.017±0.05^{b}
Ofis-96	0.016	0.020	0.018±0.11^{efg}	0.014	0.024	0.019±0.05^{b}
TMO-1	0.015	0.036	0.025±0.15$^{c\text{-}g}$	0.013	0.016	0.015±0.03^{b}
TMO-2	0.017	0.009	0.013±0.09^{fg}	0.014	0.016	0.015±0.04^{b}
TMO-3	0.005	0.013	0.009±0.07^{g}	0.015	0.019	0.017±0.06^{b}
Afyon-95	0.014	0.023	0.019±0.12^{efg}	0.013	0.015	0.014±0.03^{b}
Hüseyinbey	0.073	0.041	0.057±0.13^{abc}	0.011	0.016	0.014±0.03^{b}
Seyitgazi	0.012	0.040	0.026±0.16$^{c\text{-}g}$	0.008	0.018	0.013±0.06^{b}
Çelikoğlu	0.009	0.036	0.023±0.14$^{c\text{-}g}$	0.011	0.020	0.015±0.06^{b}
Line-8	0.058	0.047	0.053±0.13$^{a\text{-}d}$	0.025	0.017	0.021±0.09^{b}
Line-15	0.086	0.069	0.077±0.16^{ab}	0.010	0.011	0.011±0.02^{b}
Line-21	0.011	0.031	0.021±0.12$^{d\text{-}g}$	0.015	0.016	0.015±0.04^{b}
Average	0.034±0.07 B	0.037±0.05 A *		0.015±0.02 B	0.020±0.03 A**	

*,** Means followed by the same letter(s) are not different ($p\leq0.05$, $p\leq0.01$)

DISCUSSION

Capsule morphologic properties: Higher precipitation in May 2020 (69.4 mm) compared to 2021 (13.7 mm) resulted in increased stigmatic ray numbers of genotypes in the first year. A higher number of stigmatic rays is preferable to increase the number of carpels and surface area. This leads to maximum pollination, higher straw yield, and increased morphine content (Kumar and Patra, 2010).

Capsule length and diameter constitute capsule size which is the most important determinant in the alkaloid ratio (Hope *et al.,* 2020). During the first year of the experiment, high precipitation, especially before and at the beginning of inflorescence, resulted in a larger capsule diameter compared to the second year (Table 4).

Capsule dry weight has a linear relationship with latex, morphine yield per capsule, and capsule size which means that larger capsules have a greater mass of latex and morphine. It is very clear in this experiment the genotypes with large capsules had the highest capsule dry weight (Hope *et al.,* 2020). Capsule dry weight per plant showed similarities to previous studies which reported the range of capsule dry weight between 0.30-6.48 and 1.78-6.95 respectively (Karadavut and Arslan, 2006; Gümüşçü and Arslan, 1999).

The first year of the experiment showed that the capsule yield of the genotypes was low compared to the second year. The primary factor determining plant biomass yield is the number of days with active photosynthesis between full emergence and the yellowing of leaves (Acock *et al.*, 1996; Kara and Baydar, 2021). The heavy rain in the first year of the experiment mainly led to restriction to photosynthesis of poppy genotypes, leading to a decrease in capsule yield.

The results of capsule yield showed that the capsule yield of the poppy mainly depends on climatic conditions, genotype and agronomic characteristics. The previous studies reported that especially in poppy cultivation areas in Türkiye the capsule yield ranged in between 253.4 and 659.2 kg ha^{-1} (Kara and Baydar, 2021) and in between 740 to 1066 kg ha^{-1} (Valizadeh *et al.*, 2017). The results reflected that the genotypes had higher capsule yield in Ankara climatic conditions.

Phytochemical properties: Total precipitation of first year especially after January is higher compared to second year but comparing the genotypes mainly represented that while some genotypes have high morphine content in the first year some did not. This could be attributed to genetic diversity of genotypes.

Several factors influence the production of morphine, including capsule weight after seed removal, capsule size, the number of capsules per plant. Ofis-1 had

the maximum morphine ratio in both years and decreased morphine ratio observed in the second year which was statistically important. On the contrary Ofis-4 and Ofis-8 had the less morphine content in the first year of the experiment compared to second year. In the case of winter poppy cultivars, connection of frost tolerance and alkaloid accumulation is important. The frost during rosette stage can influence metabolic activities in genotypes such as alkaloid synthesis (enzymatic activity may sometimes block the morphine pathway due to low temperatures) and the tolerance of genotypes to the frost is important (Kapoor, 2020). Especially in January 2020 the average temperature is 1.4 °C and it is 2.4 °C less than January 2021. Among this autumn sown genotypes cv. Ofis-1 had shown that morphine synthesis did not damage with frost. Also, Kara (2017) reported that in some cultivars autumn sowing could lead to decrease in morphine content compared to spring sowing which is in line with our findings.

Additionally, poppy alkaloids can be transformed into each other. For example, thebaine can turn into codeine and morphine, while morphine can be chemically converted to codeine (Dittbrenner *et al.,* 2009; Singh *et al.*, 2014).

Codeine is the precursor of morphine and partial blocking of the morphine pathway, due to decreased enzymatic activity at a particular conversion step, can cause an accumulation of codeine. The biosynthesis pathway of benzylisoquinoline alkaloids is regulated by molecular, biochemical, and cellular factors (Rezaei *et al.*, 2016). It is clear that in this study environmental factors did not cause significant diversity between two years. So codeine constituents could be explained as genotypic. Gümüşçü *et al.* (2008) reported that codeine content of the poppy lines showed varieties between 0.005 to 0.270% from Türkiye. The results of this experiment revealed that codeine contents of genotypes in the average of two year showed diversity.

Conclusions: The results revealed that capsule characteristics and alkaloid content of the poppy genotypes varied among genotypes and in between years. Ankara is appropriate for poppy cultivation and the poppy capsule yield is noteworthy for Ofis-95, Çelikoğlu, Ofis-96, Hüseyinbey, Ofis-2, Ofis-3 and TMO-1 respectively. The Turkish genotypes, expected to have undergone selection in the past for opium yields, widely differed in the morphine content of their capsule husks. The genotypes in this experiment are cultivated in Ankara conditions had a morphine content between 0.32% and 1.63%. Ofis-1 and Ofis-NM had the highest morphine value and Ofis-95 had the highest capsule yield among genotypes in Ankara climatic conditions. The results favor the cultivation of these genotypes in Ankara and agronomic studies should be developed in these environmental conditions.

Conflict of interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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