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## Intraspecific variation of major alkaloids in Afghanistan opium poppy (*Papaver somniferum* L.)

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### Abstract

**Objective:** Opium poppy (*Papaver somniferum* L.) is an important source of physiologically active medicinal alkaloids. It is one of the world's best medicinal plants and the only commercial source for narcotic analgesics, morphine, codeine, the vasodilator papaverine, the cough suppressant, and the powerful anticancer drug noscapine. The current study aimed to examine the content of major alkaloids in the capsules of seven populations of poppies, collected from Balkh Province, Afghanistan.

**Methods:** Three locations (replications) were considered for each population. In each location, 15 plants with the uniform main capsules were randomly selected. A 1.5 to 2 g of capsule powder was obtained from each population/replication. Such amounts collected from three locations were then mixed to assess the major alkaloids between populations, 200 mg of which was used for extraction. Major alkaloids (morphine, codeine, thebaine, noscapine, and papaverine) were measured, using the HPLC method.

**Results:** The results indicated that the highest amount of noscapine (1398.4 µg/g DW), papaverine (802.6 µg/g DW), and thebaine (1129.2 µg/g DW) were detected in the P1 population, which appeared to be the most valuable medicinal-industrial population. For morphine and codeine, P6 (2947.7 µg/g DW) and P7 (5638.3 µg/g DW), respectively, had the highest amounts of these substances. Pharmacologically, the P4 population having the lowest morphine (narcotic drug) and the highest thebaine (non-narcotic drug) is prescribable for the medication of addicts. Monoploid genome size (2Cx DNA) was positively and significantly related to noscapine, papaverine, and codeine, indicating that a higher genome size population produced more of these alkaloids. The UPGMA phenogram constructed based on 10 variables showed four distinct cluster groups of the studied populations.

**Conclusion:** The P1 population stood out for its medicinal and industrial value due to its high contents of noscapine, papaverine, and thebaine. Conversely, The P4 population, while containing the lowest level of morphine (a narcotic drug), also had a relatively high amount of thebaine (a non-narcotic precursor to other

alkaloids). These dual-compounds metabolites make it a potentially useful population to treat drug-affected patients.

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## Introduction

Opium poppy (*Papaver somniferum* L.) is an annual plant (Bagheri *et al.* 2017), related to the Papaveraceae family and Papaveroideae subfamily. Papaveraceae is a large family of about 40 genera and 800 species that grows from tropical to alpine ecosystems (Labanca *et al.* 2018). The opium poppy is an important medicinal plant. It is a traditional source of isoquinoline alkaloids. Its dried latex contains more than 30 individual alkaloids (Kara and Baydar 2021), covering the most important commercial natural source of drugs and ornamental usage in some countries (Singh *et al.* 2014). Mainly, morphine and codeine have essential roles as analgesic drugs, thebaine as a starting material for industrial production of many pharmaceutical drugs, having non-narcotic and non-addictive nature as well as the ease of synthetic conversion to other highly-demanded drugs (Tarkesh Esfahani *et al.* 2021). Likewise, papaverine plays an important role as a coronary vasodilator, and noscapine as a potential anti-cancer and cough suppressant drug (Bagheri *et al.* 2017; Sharopov *et al.* 2018). The large diameter of its capsules is positively related to morphine's high contents (Labanca *et al.* 2018; Kara and Baydar 2018). The European Food Safety Authority introduced an acute reference dose of 10 µg morphine/kg of body weight as a safe rate for morphine in food products (Carlin *et al.* 2020). In the 21st century, the opium poppy has two main legal uses as a source of alkaloid compounds for the pharmaceutical industry and as a source of its seeds for the food industry (Gümüşçü *et al.* 2007).

Miller *et al.* (2005) reported negligible wind pollination and some insect pollination, while self-pollination was reported to be the dominant mode of opium poppy fertilization. Other reports also verified that the opium poppy is a predominantly self-pollinating species (Omidbaigi 2005; Singh *et al.* 2017; Solanki *et al.* 2017; Jesus *et al.* 2021).

The biosynthetic pathway of major alkaloids in *P. somniferum* begins with central intermediate (S)-reticuline (Ozber and Facchini 2022), leading to papaverine with two hypothetical pathways-NH pathway and NH<sub>3</sub> pathway (Han *et al.* 2010). On the other hand, the noscapine biosynthetic route comprises the embodiment of benzyloisoquinoline moiety, explained by Battersby *et al.* (1967),

through the experiment with [14C] Tyr and [14C] norlaudanosoline. Furthermore, the common pathway of three alkaloids (thebaine, codeine, and morphine) starts from tyrosine which converts to dopamine, and then to norlaudanosoline. Norlaudanosoline condenses with S-adenosyl methionine (SAM) to form reticuline. Reticuline undergoes a complex enzymatic cyclization to form salutaridine. Salutaridine is then modified through a series of steps to yield thebaine, including methylation, oxidation, and the rearrangement of the carbon skeleton. Thebaine can be converted to codeine by the enzyme thebaine 6-O-demethylase, which removes a methyl group from a specific position on the molecule (Unterlinner *et al.* 1999). Finally, codeine is converted to morphine by the enzyme codeine demethylase, which removes another methyl group (Hagel and Facchini 2010; Onoyovwe *et al.* 2013; Vadhel *et al.* 2023). In summary, morphine, thebaine, and codeine together share the most similar pathways, while papaverine and noscapine have somewhat separate routes with (S)-reticuline as the starting point.

There are several techniques available for the analytical study of *P. somniferum* alkaloids such as TLC (Pothier and Galand 2005), HPLC with massive diversity of detection methods (Sankar *et al.* 2019, 2020; Liang *et al.* 2022), LC-MS (Carlin *et al.* 2020), UV, DAD, or fluorescence methods, GC, GC-MS, HPLC coupled with MS (HPLC-MS), CE-MS, 2D LC, and IMS (Filipiak-Szok *et al.* 2018; Tajabadi *et al.* 2022). According to the research, there is no comprehensive study related applying the HILIC method for the separation and isolation of opium alkaloids (Bagheri *et al.* 2017). To select the best column for analytical purposes, bare silica, and zwitterionic stationary phases were recommended (Bagheri *et al.* 2017). In the present study, our goal was to use the HILIC method by the bare silica column to determine the major alkaloids of opium poppy.

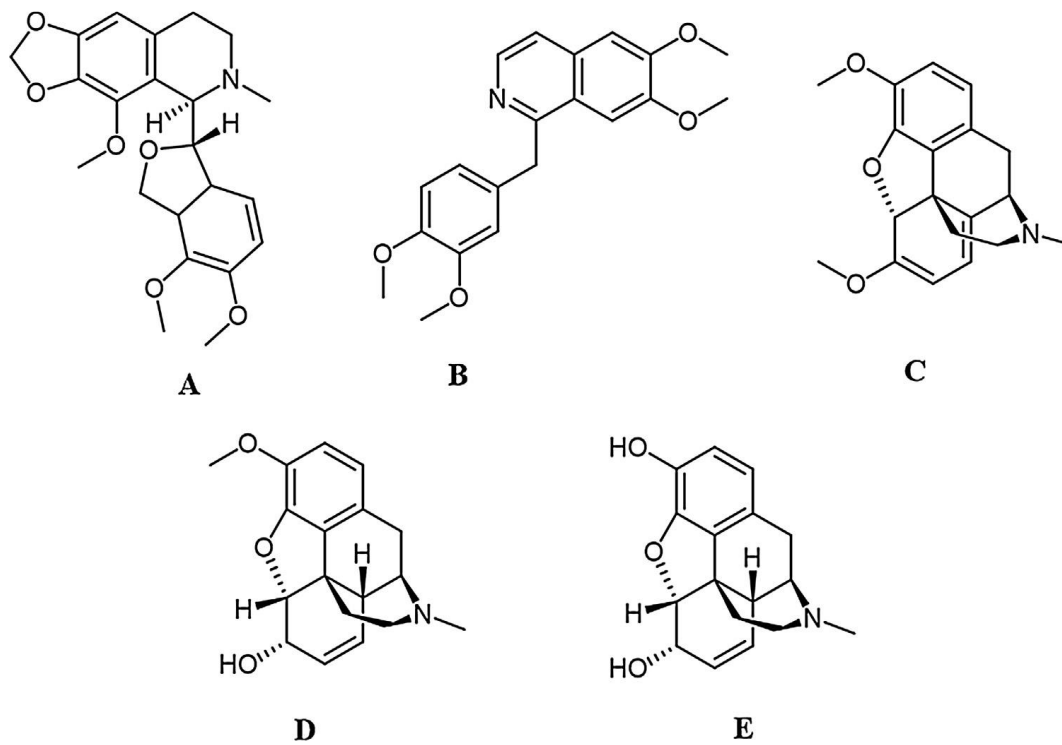
## Materials and Methods

The five major alkaloids (Figure 1) reference standards of morphine, codeine, thebaine, noscapine, and papaverine were purchased from Temad Co. Darou Pakhsh (Tehran, Iran). Acetonitrile (ACN) of HPLC gradient grade was obtained from Chem-Lab NV (Zedelgem, Belgium). The ultra-pure water was provided by the Millipore Milli-Q system (Billerica, MA, USA) and used throughout the experiment. All stock solutions were kept at 4 °C. Seven populations of *P. somniferum* L. were collected from seven different districts of Balkh Province, Afghanistan (Table 1, Figure 2). The shade drying method was applied to the collected ripped capsules and then the seeds were removed. Three locations (replications) were considered for each population. We reviewed previous similar studies and took into account their methodologies. Peer-reviewed evidence supports the use of single replicates in HPLC experiments for practical reasons like cost, time constraints, or sample

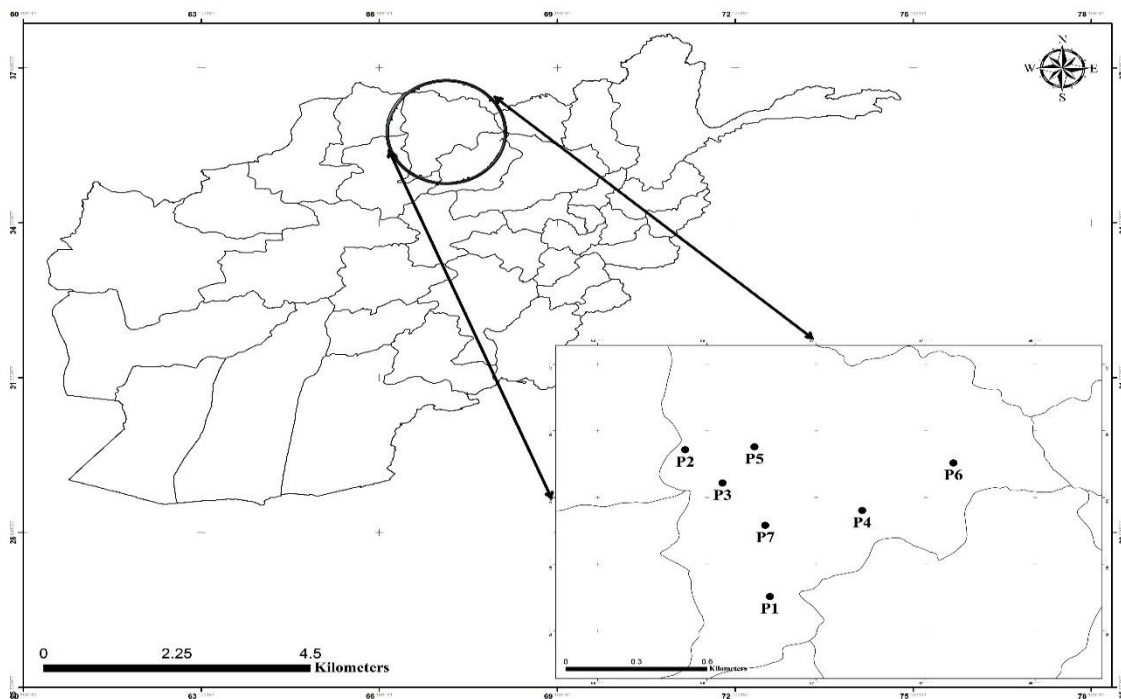
availability. In such studies, all replications are mixed and ultimately one replicate is used for each population (Kumar *et al.* 2016; Ganorkar and Shirkhedkar 2017; Abdollahi *et al.* 2020). Hence, in the current study, in each location, 15 plants with uniform main capsules were randomly selected. A 1.5 to 2 g of capsule powder was obtained from each replication per population and then, mixed (4.5-6 g) to assess the major alkaloids in the populations. A power analysis was performed to determine the number of replications required to achieve a significance level of 0.05 with a power greater than 0.8 (Garland-Campbell 2018). An amount of 200 mg of pooled capsule powder from each population was considered for extraction for injecting into HPLC, using three technical replications per population for statistical analysis. Twenty ml of methanol (MeOH, HPLC grade) solvent (Merk, Darmstadt, Germany) was added to dissolve all chemical and physicochemical compounds. It was placed in power sonic 505 (Seoul, Korea) for 2 h. The sample was then placed in the reflux oil bath at the MeOH boiling point temperature (65 °C) for the next 2 h. Finally, after the filtration of the sample, for evaporation of the solvent, the rotary was used and the extract was kept at 4 °C until the three injections at HPLC. Ammonium acetate buffer (60 mM) was prepared in the purified water and the pH was subsequently adjusted to 3, using glacial acetic acid. The mobile phase was implemented for the simultaneous separation of opium alkaloids on Bare Silica (250 × 4.6 mm dimensions) with 10 µm particle size, 100 pore size (Å), and 300 surface area (m<sup>2</sup>/g). The HILIC column was comprised of ammonium acetate buffer (solvent A) and ACN (solvent B) (Acevska *et al.* 2014). The five major alkaloids were separated, using the gradient elution as follows: 0 to 5% of solvent A in 10 min, and 95% of solvent B at a same time. Reaching 15% within 25 min solvent A was equal to 85% solvent B. Finally, solvent A reached 85% in 75 min, and solvent B reached 15% at the same time. The 20 µl injection was performed with a flow rate of 0.7 ml/min (Bagheri *et al.* 2017). Stock solutions were

**Table 1.** Collected locations of *Papaver somniferum* populations of Afghanistan, Balkh Province.

Population codes	Locality	Latitude (N)	Longitude (E)	Altitude (m)
P1	Chahar Kint	36°20'33.43"	67°31'23.38"	1822
P2	Balkh	36°45'54.62"	66°53'19.24"	341
P3	Kishindeh	35°48'11.83"	67°05'38.92"	1862
P4	Chimtal	36°28'27.42"	66°57'3.47"	553
P5	Khulm	36°42'50.09"	66°57'7.92"	365
P6	Sholgara	36°22'12.24"	66°53'29.67"	551
P7	Chahar Bolak	36°48'5.64"	66°57'3.68"	335



**Figure 1.** Chemical structures of five opium poppy (*Papaver somniferum* L.) major alkaloids, Noscapine (A), papaverine (B), thebaine (C), codeine (D), and morphine (E).



**Figure 2.** Geographic distribution of collected *Papaver somniferum* populations on the map of Afghanistan, using ArcGIS.

prepared at the concentrations of 5 mg/ml for morphine, codeine, thebaine, and papaverine, and 2.5 mg/ml for noscapine in the acetonitrile/deionized water solvent mixture (95:5, v/v). These stock solutions were stored at 4 °C. Working solutions for the analytical HPLC were prepared from a suitable amount of the preliminary stock solutions and were diluted by the acetonitrile/deionized water solvent mixture. A Knauer HPLC system (Berlin, Germany) with EZChromElite software was used to monitor and acquire the chromatographic data. The column was maintained at room temperature and sample compounds were detected at a wavelength of 280 nm.

The normality test was first carried out on the residuals, using Minitab 17. Log 10 transformation was used for the noscapine data, while NScor transformation was used for the other four alkaloids' data. Analysis of variance (ANOVA) was done based on a completely randomized design with three technical replicates, using the General Linear Models (GLM) in SAS 9.1 software. Mean comparisons were performed, using the least significant difference test. Moreover, a cluster analysis was carried out on cytogenetic characteristics (chromosome length and monoploid genome size; Rasekh and Karimzadeh 2023), geographic variables (longitude, latitude, and altitude), and major alkaloids' amounts (noscapine, papaverine, thebaine, codeine, and morphine), using Minitab 17 software, through the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method and the Euclidean distance ( $r = 0.985$ ), using NTSYSpc ver. 2.10e to assess similarities and variations among the populations. Also, the Pearson correlation was calculated among the above-mentioned 10 variables. Considering the three different biosynthesis pathways in papaverine, noscapine, and the other three alkaloids (morphine, thebaine, and codeine), correlation coefficients were separately calculated. The amount of alkaloids was also regressed upon the genome size, using a linear regression equation.

## Results

Different parts (capsule, stem, leaf, and root) of opium poppy plants were first considered for the morphinane alkaloids' content. Of those, the capsules appeared to be the best for accumulating the highest content of alkaloids. Hence, the capsules' alkaloids were considered in the current study. Sharopov *et al.* (2018) also emphasized a common source of morphinane alkaloids in the capsules of poppy plants. In the present report, ANOVA showed statistically significant ( $p < 0.01$ ) differences among the opium poppy populations from the Balkh Province, Afghanistan (Table 2), indicating an intraspecific variation for all five major alkaloids. Between-population mean comparisons (Table 3) showed remarkable differences among populations for each major alkaloid. The means of major

**Table 2.** Analysis of variance of five major alkaloids of *Papaver somniferum* populations of Afghanistan, Balkh Province.

SOV	df	Mean squares				
		Noscapine	Papaverine	Thebaine	Codeine	Morphine
Populations	6	0.49512**	2.9778**	2.9771**	2.9778**	2.9778**
Error	14	0.00000061	0.0531	0.0509	0.0531	0.0531
CV%	-	0.03	7.68	7.52	7.68	7.68

\*\* Significant difference ( $p < 0.01$ ).

**Table 3.** Means ( $\pm$  SE) of five major alkaloids in *Papaver somniferum* populations of Afghanistan, Balkh Province.

Populations	Noscapine ( $\mu\text{g/g DW}$ )	Papaverine ( $\mu\text{g/g DW}$ )	Thebaine ( $\mu\text{g/g DW}$ )	Codeine ( $\mu\text{g/g DW}$ )	Morphine ( $\mu\text{g/g DW}$ )
P1	1398.40 <sup>a</sup> $\pm$ 1.06	802.60 <sup>a</sup> $\pm$ 4.890	1129.20 <sup>a</sup> $\pm$ 12.500	1243.00 <sup>b</sup> $\pm$ 0.979	1948.40 <sup>b</sup> $\pm$ 16.00
P2	707.24 <sup>b</sup> $\pm$ 1.13	49.44 <sup>cd</sup> $\pm$ 0.077	251.89 <sup>e</sup> $\pm$ 00.020	474.11 <sup>cd</sup> $\pm$ 0.034	1294.30 <sup>cd</sup> $\pm$ 00.05
P3	275.33 <sup>e</sup> $\pm$ 0.01	45.98 <sup>ed</sup> $\pm$ 0.117	283.24 <sup>de</sup> $\pm$ 02.450	98.23 <sup>f</sup> $\pm$ 0.076	293.18 <sup>e</sup> $\pm$ 00.44
P4	430.51 <sup>d</sup> $\pm$ 0.06	53.29 <sup>bc</sup> $\pm$ 0.054	1099.02 <sup>b</sup> $\pm$ 02.100	530.30 <sup>bc</sup> $\pm$ 0.260	278.15 <sup>f</sup> $\pm$ 00.05
P5	145.31 <sup>f</sup> $\pm$ 0.04	24.76 <sup>f</sup> $\pm$ 0.001	980.95 <sup>bc</sup> $\pm$ 00.000	443.70 <sup>de</sup> $\pm$ 0.004	697.75 <sup>ed</sup> $\pm$ 00.57
P6	98.42 <sup>g</sup> $\pm$ 0.20	24.31 <sup>e</sup> $\pm$ 0.001	338.48 <sup>cd</sup> $\pm$ 00.005	391.96 <sup>e</sup> $\pm$ 0.850	2947.70 <sup>a</sup> $\pm$ 01.00
P7	656.27 <sup>c</sup> $\pm$ 0.16	162.88 <sup>b</sup> $\pm$ 0.432	42.12 <sup>f</sup> $\pm$ 00.007	5638.3 <sup>a</sup> $\pm$ 0.180	1722.30 <sup>bc</sup> $\pm$ 04.30
Mean	530.21	166.47	589.31	1259.94	1311.68
Range	98.42-1398.40	24.76-802.60	42.122-1129.20	98.23-5638.3	278.15-2947.7
LSD <sub>1%</sub>	0.002	0.56	0.548	0.56	0.56

alkaloids for all seven opium poppy populations were as follows (Table 3): noscapine (530.21  $\mu\text{g/g DW}$ ), papaverine (166.47  $\mu\text{g/g DW}$ ), thebaine (589.31  $\mu\text{g/g DW}$ ), codeine (1259.94  $\mu\text{g/g DW}$ ), and morphine (1311.68  $\mu\text{g/g DW}$ ). The highest amounts of noscapine (1398.4  $\mu\text{g/g DW}$ ), papaverine (802.6  $\mu\text{g/g DW}$ ), and thebaine (1129.2  $\mu\text{g/g DW}$ ) were detected in the P1 population. For morphine and codeine, P6 (2947.7  $\mu\text{g/g DW}$ ) and P7 (5638.3  $\mu\text{g/g DW}$ ), respectively, appeared to have the highest amounts (For HPLC chromatograms refer to Supplementary Figure 1).

The UPGMA phenogram constructed based on similarities of cytogenetic traits, geographic variables, and major alkaloid amounts (Figure 3) showed four major clusters. P1 population was located in the 1st cluster, four populations (P2, P4-P6) were located in the 2<sup>nd</sup> cluster, while P3 and P7 were placed in the other two separate clusters.

Noscapine, papaverine, and codeine alkaloids were positively and significantly ( $p < 0.01$ ) related to the monoploid genome size (Tables 4, 5; Figure 4). In other words, these three alkaloids

accumulated more in the capsules of all *P. somniferum* populations as their genome sizes increased. Altitude also positively and significantly ( $p < 0.05$ ) correlated with thebaine (Table 5).

**Table 4.** Pearson correlation coefficients of noscapine and papaverine alkaloids with cytogenetical and geographical characteristics in *Papaver somniferum* populations of Afghanistan, Balkh Province

Alkaloids	CL ( $\mu\text{m}$ )	GS (pg)	Altitude	Latitude	Longitude
Noscapine	-0.139 <sup>ns</sup>	0.924 <sup>**</sup>	0.301 <sup>ns</sup>	0.142 <sup>ns</sup>	0.591 <sup>ns</sup>
Papaverine	-0.009 <sup>ns</sup>	0.934 <sup>**</sup>	0.367 <sup>ns</sup>	-0.005 <sup>ns</sup>	0.631 <sup>ns</sup>

<sup>ns, \*\*</sup> Non-significant ( $p > 0.05$ ) and significant difference ( $p < 0.01$ ), respectively; CL: Chromosome length; GS: Genome size.

**Table 5.** Pearson correlation coefficients of thebaine, codeine, and morphine alkaloids with cytogenetical and geographic characteristics in *Papaver somniferum* populations of Afghanistan, Balkh Province

Alkaloids	CL ( $\mu\text{m}$ )	GS (pg)	Codeine	Morphine	Altitude	Latitude	Longitude
Thebaine	-0.751 <sup>**</sup>	0.146 <sup>ns</sup>	-0.060 <sup>ns</sup>	-0.065 <sup>ns</sup>	0.443 <sup>*</sup>	-0.300 <sup>ns</sup>	0.634 <sup>ns</sup>
Codeine	0.427 <sup>ns</sup>	0.760 <sup>**</sup>	1	0.126 <sup>ns</sup>	-0.291 <sup>ns</sup>	0.648 <sup>ns</sup>	-0.216 <sup>ns</sup>
Morphine	0.269 <sup>ns</sup>	0.247 <sup>ns</sup>	--	1	0.011 <sup>ns</sup>	0.129 <sup>ns</sup>	0.187 <sup>ns</sup>

<sup>ns, \*, \*\*</sup> Non-significant ( $p > 0.05$ ) and significant differences ( $p < 0.05$ ,  $p < 0.01$ ), respectively.

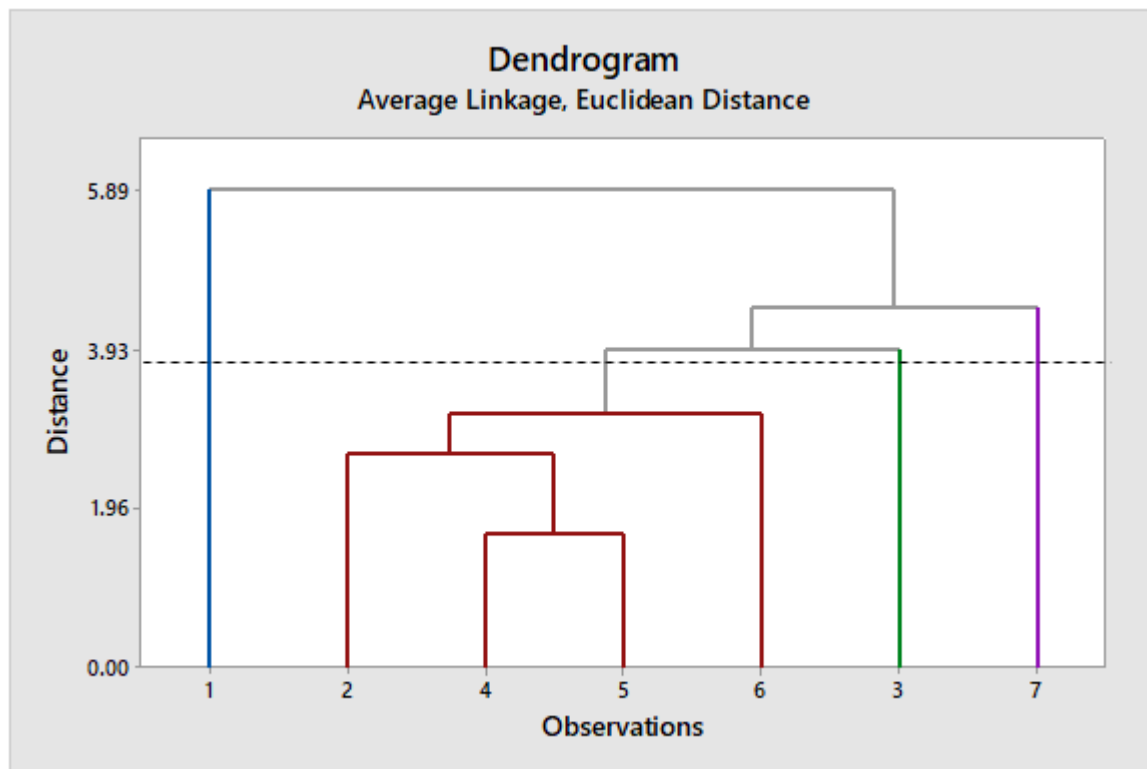
## Discussion

During three years (2005-2007), 300 accessions of (*P. somniferum* L.) were examined for five major alkaloids (morphine, codeine, thebaine, papaverine, and noscapine), analyzed by HPLC in Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany (Dittbrenner *et al.* 2009), in which, the 3-year codeine content in the 40 selected accessions was 1800.6  $\mu\text{g/g}$  DW in 2005, 1360.7  $\mu\text{g/g}$  DW in 2006, and 1432.9  $\mu\text{g/g}$  DW in 2007. In the present study, the average codeine amount of the seven populations of opium poppy of Balkh-Afghanistan was 1259.9  $\mu\text{g/g}$  DW, ranging from 98.23 (P3) to 5638.3  $\mu\text{g/g}$  DW (P7), showing 18% lower compared to that reported by Dittbrenner *et al.* (2009). Interestingly, the highest amount in our study was for the P7 population (5638.3  $\mu\text{g/g}$  DW), showing 3.7-fold higher in codeine than that in the 3-year average of all accessions (1531.4  $\mu\text{g/g}$  DW) studied by Dittbrenner *et al.* (2009). The same extent was not true of the other four (noscapine, papaverine, thebaine, and morphine) alkaloids in our study.

*NCS* and *SAT* were reported as two important genes with significant contributions in regulating the thebaine content of diploid and induced tetraploid *Papaver bracteatum* Lindl. (Tarkesh Esfahani *et al.* 2021). Hence, the *NCS* and *SAT* genes were enhanced remarkably in the leaves of the tetraploids (6.6-fold and 13.5-fold, respectively) at the transcription level compared to those of diploids that had a thebaine content of 6.9  $\mu\text{g/g}$  DW (Tarkesh Esfahani *et al.* 2021). Compared to the thebaine content of the Iranian diploid *P. bracteatum* Lindl. (6.9  $\mu\text{g/g}$  DW), the diploid Afghan *P. somniferum*



populations evaluated in the present study were 85-fold higher (589.31  $\mu\text{g/g DW}$ ) ranging from 42.12  $\mu\text{g/g DW}$  (P7) to 1129.20  $\mu\text{g/g DW}$  (P1), proving a great superiority of Afghan opium poppy.



**Figure 3.** Dendrogram showing the phenetic relationships among seven *Papaver somniferum* populations ( $r = 0.985$ ).

Gene expression analysis through semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) has exhibited that the transcription level of *SAT* gene (thebaine producer) of the benzylisoquinoline biosynthesis pathway increased 2.2-fold in the tetraploid plants in comparison to that in the controls (diploid) plants (Madani *et al.* 2019). Thus, *P. bracteatum* has enormous potential for synthesizing anti-addiction compounds such as thebaine (8.8  $\mu\text{g/g DW}$  compared with 1.5  $\mu\text{g/g DW}$  in the controls (5.86-fold higher) (Madani *et al.* 2019). Induced tetraploids have shown a substantial increase in thebaine in both reports of Madani *et al.* (2019) and Tarkesh Esfahani *et al.* (2021). However, shockingly we observed the mean content of 589.3  $\mu\text{g/g DW}$  for thebaine from the seven populations of opium poppy of Balkh-Afghanistan in the current study without using any elicitors or inducing tetraploid. In our study, the thebaine content was 85.4-fold higher than that of the Iranian diploid, *P. bracteatum*, reported by Tarkesh Esfahani *et al.* (2021) and 66.8-fold higher than in the induced tetraploids of Iranian poppy reported by Madani *et al.* (2019).

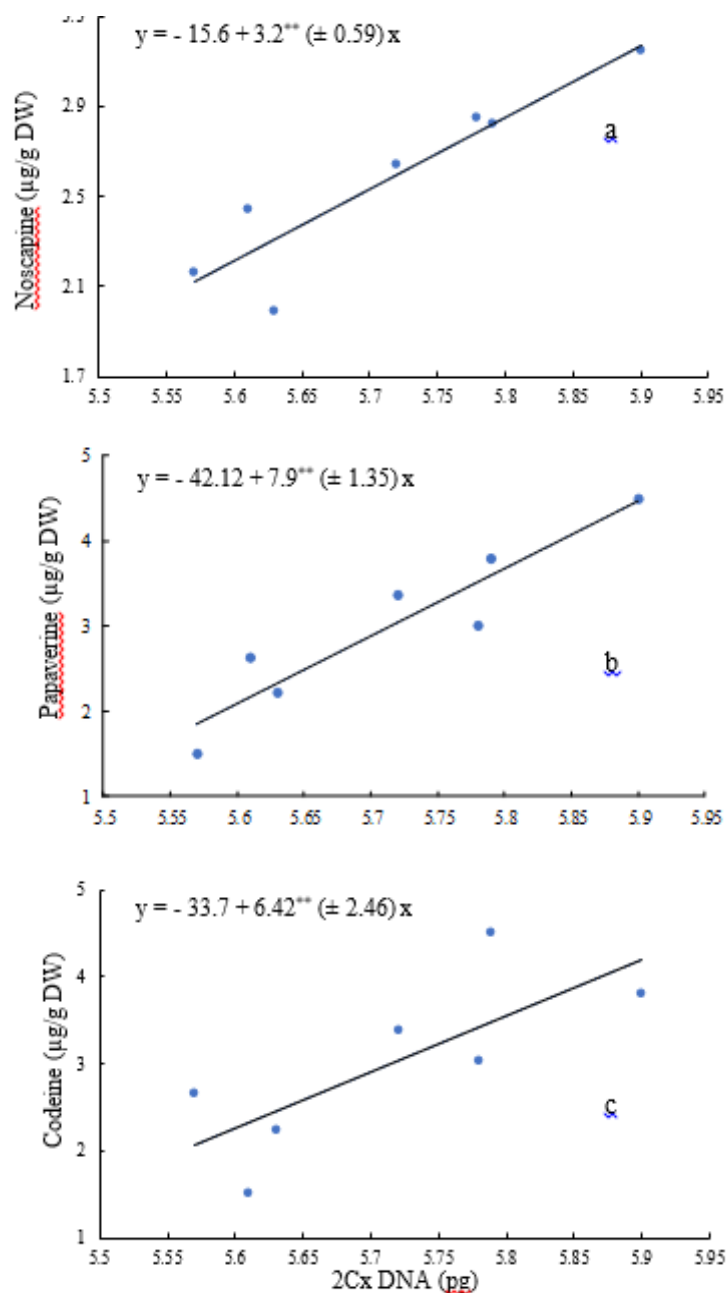
Different abiotic elicitors play a significant role in improving the production of different secondary metabolites. Among which, methyl jasmonate (MeJA) is capable of enhancing the amounts

of various alkaloids in different plants (Cho *et al.* 2007; Holková *et al.* 2010; Mishra *et al.* 2013; Akhgari *et al.* 2019; Tarkesh Esfahani *et al.* 2021; Santos *et al.* 2022). The effects of induced MeJA elicitation on the root production of thebaine in Iranian endemic *P. bracteatum* showed that the highest thebaine content (9.3 µg/g DW), compared to that in the non-treated diploid controls (6.8 µg/g DW; 1.4-fold increase), identified in the plants treated by 0.5 mM MeJA (Tarkesh Esfahani *et al.* 2021). In the present study, the mean content of thebaine was 589.3 µg/g DW in all seven populations of opium poppy of Balkh-Afghanistan, indicating a 63.4-fold increase compared to that in the induced 0.5 mM MeJA elicitation (9.3 µg/g DW) of *P. bracteatum* report (Tarkesh Esfahani *et al.* 2021).

In the current study, among seven *P. somniferum* populations studied, the P1 population showed 1129.2 µg/g DW thebaine, proving a 121-fold increase rather than *P. bracteatum* report. Therefore, from an economic perspective, the P1 population seems to be strategically the most valuable, exceptional, and irreplaceable. Madani *et al.* (2019) confirmed that an induced tetraploid plant had 30.55-fold higher noscapine content (4677.4 µg/g DW) compared to the control (diploid) plants (153.8 µg/g DW). The same comparison was approved in gene expression analysis for *TYDC* (noscapine producer gene) with about a 2-fold increase.

The alkaloid composition of different parts of *P. somniferum* was investigated by Sharopov *et al.* (2018) and this report for capsule alkaloid content indicates morphine, codeine, and papaverine 929.3 µg/g DW, 1280.5 µg/g DW, and 2062.9 µg/g DW, respectively. The grand mean of morphine alkaloid of seven populations in the current study was obtained as 1311.7 µg/g DW (278.2-297.7 µg/g DW) which was 1.4-fold higher than the Sharopov *et al.* (2018) report. Following that, the grand mean of codeine of seven studied populations was 1259.9 µg/g DW, ranging from 98.23-5638.3 µg/g DW which shows the same story in both reports. The comparison of the current study with the above report indicates that the P7 population with 5638.3 µg/g DW codeine content had a 4.4-fold higher amount of this metabolite and the P6 population with 2947.7 µg/g DW morphine content possessed 3-fold higher amounts. However, in the case of papaverine, the P1 Afghan population showed a 2.5-fold lower amount. Furthermore, the study conducted by Abedini *et al.* (2018) regarding the transcriptome data of eight genera of the Papaver family (Papaveraceae), revealed that noscapine biosynthesis genes are present in the Iranian poppy (*P. bracteatum*), while they do not exist in other genera of this family. Thus, in agreement with Abedini *et al.* (2018), in the current report, the uppermost amount of noscapine in the P1 Afghan population (the most southern latitude, Table 1, Figure 1) was 1398.4 µg/g DW, indicating the activity of the genes of this alkaloid in this species.

In the study of Gümüşçü *et al.* (2007) 99 lines out of 1000 lines of the opium poppy plant



**Figure 4.** Linear regression equation of noscapine (a), papaverine (b), and codeine (c) with monoploid genome size ( $2Cx$  DNA,  $\text{pg}$ ) in *Papaver somniferum*.

(*P. somniferum*) were selected in Turkey. The major alkaloids noscapine, papaverine, thebaine, codeine, and morphine were reported to be 0.099%, 0.029%, 0.041%, 0.049%, and 0.475%, respectively. Compared to Afghan opium, this study found a significantly lower content of codeine and morphine (252 and 28-fold lower, respectively). In other words, the major alkaloids of Balkh, Afghanistan opium show a good performance, most likely indicating the genetic and environmental potential of this country compared to the populations and the environment of Turkey, reported by Kara and Baydar (2021).

## Conclusion

The P1 population of *P. somniferum* stands out for its medicinal and industrial value due to its high contents of the valuable alkaloids noscapine, papaverine, and thebaine. Conversely, the P4 population, while containing the lowest level of morphine (a narcotic drug), also has a relatively high amount of thebaine (a non-narcotic precursor to other alkaloids). These dual-compounds metabolites make it a potentially useful population to treat drug-affected patients.

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## Ethical considerations

The authors avoided data fabrication and falsification.

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## Conflict of interest

The authors declare that they have no conflict of interest with any organization concerning the subject of the manuscript.

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