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Original Article

# Evaluation the Variation of Morphine Percentage in Different Turkish Opium Poppy (*Papaver somniferum* L.) Lines in Three Years

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## Abstract

Opium poppy (*Papaver somniferum* L.) has two major products: alkaloids in the capsules and the seeds. It is known that opium poppy today contains alkaloid in great quantities. Opiate alkaloids and their synthetic derivatives are widely used in medicines which are produced in hundreds of tons for the medicine industry. The study aimed to screen the capsules of six Turkish opium poppy lines to evaluate their morphine percentage. The trial was carried out at the Experimental Fields of the Agronomy Department, Faculty of Agriculture of Ankara University, Turkey during three years. All seeds were sown during first week of October and harvested during second week of July during three years of experimentation to investigate the variation of morphine content. During first year hairless stem and strong capsule types were selected and sown followed by. Alkaloid analysis was performed at the laboratory of the alkaloid factory at Bolvadin province of Turkey in three years. Morphine percentage of six different lines were determined. The results showed that the average of morphine contents of lines ranged 0.731-0.851%, 0,813-1,147% and 0.815-1.142% during 2010, 2011 and 2012 respectively. In conclusion, these opium poppy especially $L_1$ and $L_2$ lines could serve as major source of morphine in future.

**Key words:** Alkaloid, Morphine, Poppy, *Papaver somniferum*

## Introduction

Opium poppy (2n=22) of the family *Papaveraceae* is one of the few herbs of temperate and subtropical climate that is being grown since centuries around the world for its medicinal properties [1]. It is a plant known to produce more than 80 alkaloids [2]. Pharmaceutically important alkaloids include the analgesic morphine, codeine, antispasmodic papaverine, etc. [3]. The plant continues to synthesize morphine from the seedling stage to its maturity. While the morphine concentration in young plants is higher in the root than shoot, it is relatively higher in the leaves and stem than roots in plants approaching an thesis [1]. With the onset of capsule development, morphine becomes concentrated in the peduncle and capsule. The harvestable morphine in the capsule and peduncle is a product of alkaloid metabolism in the entire laticiferous system of the plant.

Poppy growing areas of the world have ranged from Bombay to Moscow in the Northern hemisphere and to Tasmania in the Southern hemisphere. Major poppy growing countries of the world were Turkey, India, Japan, China, Australia, France and Spain. Turkey is accepted as a traditionally poppy growing country by the United Nations. Poppy has been grown in Anatolia since Hittites. It was one of the main incomes during the Ottoman Empire. In the beginning, poppy planting and opium production were regulated without any restriction. However, after the foundation of Turkish Republic in 1923, governmental rules were accepted in 1933 and the poppy has become a plant controlled by the state and has been accepted as a narcotic drug.

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Turkish Grain Board (TMO) was founded in 1938 and has undertaken the control of growing, products purchase, and storage and trade of poppy plant ever since. Dry capsules have been used for extracting alkaloids in Turkey since 1974 whereas India is the only country that legally produces opium by incising the unripe capsule [4]. Utilization of dry capsules is an advantage for production of alkaloids. Because, unripe capsules can be used illegally but dry capsules can no longer become a raw material for illicit purposes. Today, the plant is cultivated to obtain dry capsule for the production of morphine and its derivatives in the alkaloid factory located at Bolvadin province of Turkey. The opium poppy plant was grown under control and the capsules of plants were purchased by the government. Factory was designed mainly to produce morphine, but some other alkaloids production was also practiced [5,6]. In recent years the global trends show that the consumption of opium alkaloids and its derivatives are growing. Morphine, which is the main and narcotic component of opium, showed a tenfold demand in the last two decades. Despite the important role of this crop in some countries economy, a high yielding variety for high latex (opium) and seed yield with specific alkaloids has not been developed [7,8].

Plant breeding is said to be both an art and a science [9,10]. Crop breeding included domestication of plants, selection, and hybridization. Domestication is a selection process used to adapt plants to better suit the needs of humans. The process of domestication has been going on for 10,000 years [11]. During domestication, selection was practiced for traits such as increased seedling vigor, increase in seed number, reduction in toxic chemicals, etc [12]. The existence of substantial variations in the available gene pool of a species is necessary for any successful breeding programme. Several independent studies on the evaluation of the genetic variation in the cultivated germplasm of *P. Somniferum* reached the conclusion that only a limited variation prevails in Indian genetic stocks [7,13] and European stocks [14] as Turkey for most agronomic and chemical traits. Varietal, mass and pure line selections have been applied by several breeders of opium poppy for the development of improved cultivars [15,16].

However, the most widely used method which has produced several commercial cultivars is the pedigree selection by which, through hybridization between parents with different desirable characteristics, lines combining most of them are developed. The pedigree method has been used successfully for increasing the yield of capsules, opium and seeds and the morphine content .Substantial progress has been achieved during the last 30 years in France, where the yield of morphine has increased from 4.5 kg/ha in 1961 to 10.5 kg/ha in 1991. This has been achieved mainly through genetic and, to some extent, agro-technical improvements of the morphine content of capsules [14].

Recurrent selection ensuring the renewal of a larger genetic basis than the pedigree method has been suggested for the improvement of the agronomic and chemical characteristics of opium poppy [14,16]. The production of synthetic cultivars increases the genetic basis of the population [17].

**Fig. 1** Strong (left) and weak (right) capsules of Turkish opium poppy lines

**Fig. 2** Hairless (left) and hairy (right) stems of Turkish opium poppy lines

The success of any crop improvement program depends mainly on a judicious selection of promising parents from gene pools. There are different types of capsule (strong and weak) and stem (hairy and hairless) among Turkish opium poppy lines (Fig. 1,2.).

Judicious selection of strong capsule and hairless stem types among the plants of Turkish opium poppy lines and evaluation the percentage of morphine in capsules can prepare background as substances; follow by commercial cultivars will be produced by the use of these substances in different breeding methods. The aim of this study was to evaluate selected ones (strong capsule and hairless stem) among the plants of six Turkish opium poppy lines for morphine percentage trait.

## Material and Method

The trial was carried out at the experimental fields of the Agronomy Department, Agriculture Faculty of Ankara University, Ankara, Turkey, during three agricultural seasons (2009-2010, 2010-2011 and 2011-2012). The climatic data of the experimental city (Table 1.) and soil analysis results of the experimental soil samples in the field (Table 2.) are shown.

Six Turkish opium poppy lines were used in this study. The materials were token from opium poppy collections, genetic stocks in the Agronomy Department Faculty of Agriculture, Ankara University, Ankara. Some characteristics of lines are summarized in Table 3.

All materials were sown in 4 rows as autumn planting during first week of October each year. Sowing was performed as 30 x 10 cm of plant spacing. Each plot was 1.2 m × 4 m = 4.8 m2. Normally fertilization and irrigation were applied to the experiment. During first year hairless stem and strong capsule types of six Turkish opium poppy lines were marked.

Capsules of marked plants were harvested in the plots during second week of july 2010 and sown followed by. Morphine percentage of capsules was analyzed any year and the variation of morphine percentage determined. Morphine Analyze: After harvesting, capsules of different opium poppy lines were ground and sent to the alkaloids factory in Bolvadin-Afyon province.

The content of morphine in the samples (dry mass of capsules) was investigated at the laboratory of the factory using HPLC. HPLC conditions were: column, 300 mm £ 3.9 mm Bundapak C18; Xow, 1.5 ml/min; standard, 0.1 mg/ml [6] and HPLC 1050. A weight of 8.2 g NaAc is dissolved in 2 l distilled water and then Wltered on the Wlter paper. pH is calibrated to 3.6 with glacial acetic acid. To a volume of 1,760 ml of this solution, 180 ml acetonitril, 60 ml ethanol and 60 ml THF were added. Content determination process was performed. The results were presented in a descending order according to the morphine rate in the samples.

**Table 1** Outdoors climatic data of the experimental city*

| | Rainfall (mm) | | | | Temperature (ºC) | | | | Humidity | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Months | Long term | 2009-2010 | 2010-2011 | 2011-2012 | Long term | 2009-2010 | 2010-2011 | 2011-2012 | Long term | 2009-2010 | 2010-2011 | 2011-2012 |
| September | 17.5 | 10.3 | 1.5 | 0.6 | 18.7 | 18.5 | 22.5 | 20.1 | 48.7 | 48.6 | 42.3 | 42.4 |
| October | 33.2 | 13.7 | 167.6 | 62.4 | 13.0 | 16.6 | 12.2 | 10.9 | 62.1 | 49.2 | 72.3 | 65.1 |
| November | 35.4 | 43.1 | 32.0 | 10.9 | 6.7 | 7.4 | 11.2 | 3.3 | 72.7 | 74.6 | 63.6 | 70.3 |
| December | 42.5 | 68.0 | 67.3 | 39.3 | 2.3 | 5.4 | 6.1 | 3.8 | 77.8 | 78.5 | 78.8 | 74.9 |
| January | 39.2 | 63.0 | 42.0 | 93.3 | 0.3 | 3.1 | 2.4 | -0.9 | 77.9 | 77.8 | 78.5 | 87.1 |
| February | 33.4 | 65.1 | 24.3 | 47.7 | 2.1 | 6.5 | 3.2 | -1.9 | 72.1 | 70.4 | 69.8 | 83.7 |
| March | 36.7 | 44.6 | 57.5 | 43.0 | 6.2 | 8.5 | 6.0 | 3.7 | 63.2 | 59.9 | 67.1 | 67.2 |
| April | 50.0 | 37.5 | 50.1 | 24.8 | 11.3 | 12.2 | 10.0 | 14.9 | 60.2 | 54.4 | 65.6 | 50.5 |
| May | 50.3 | 31.0 | 73.1 | 65.1 | 16.1 | 18.4 | 15.2 | 17.5 | 57.6 | 44.4 | 62.3 | 56.8 |
| June | 35.3 | 57.8 | 44.4 | 39.8 | 20.2 | 21.5 | 19.7 | 20.1 | 52.7 | 54.2 | 55.4 | 54.7 |
| July | 15.5 | 25.7 | 10.7 | 13.2 | 23.6 | 26.2 | 25.6 | 24.9 | 45.2 | 44.2 | 42.7 | 43.3 |
| August | 12.0 | 0.4 | 21.1 | 8.9 | 23.3 | 28.4 | 23.8 | 25.6 | 44.2 | 30.5 | 44.9 | 42.4 |

* The government meteorological association of Turkey

**Table 2** Soil analyses results of the experimental soil samples in the field in three years

| Year | 2009-2010 | 2010-2011 | 2011-2012 |
|---|---|---|---|
| pH | 7.85 | 7.9 | 7.57 |
| $CaCO_3$ (kg/da) | 9.00 | 8.35 | 11.07 |
| Organic Material (%) | 1.25 | 1.09 | 1.10 |
| Organic Carbon (%) | 0.61 | 0.62 | 0.59 |
| $P_2O_5$ (kg/da) | 7.85 | 6.42 | 5.30 |
| $K_2O$ (kg/da) | 160 | 116 | 122 |
| Salt (%) | 0.085 | 0.043 | 0.062 |
| Texture | Clay-Loam | Clay-Loam | Clay-Loam |
| EC (dS/m) | 1.162 | 1.083 | 1.144 |
| B.S.P | 56 | 62 | 58 |

**Table 3** Some identification characteristics of opium poppy seeds in different lines

| Line No. | Seed color | Flower color |
|---|---|---|
| $L_1$ | Grey | Light Violet |
| $L_2$ | Grey | Light Violet |
| $L_3$ | Grey | Light Violet |
| $L_4$ | Pink | Violet |
| $L_5$ | Pink | Violet |
| $L_6$ | Pink | Violet |

**Table 4** Averages of morphine percentage in different lines (dry capsules)

| Line No. | 2009-2010 | 2010-2011 | 2011-2012 |
|---|---|---|---|
| $L_1$ | 0.745 | 1.147 | 1.142 |
| $L_2$ | 0.851 | 1.015 | 1.063 |
| $L_3$ | 0.737 | 0.939 | 0.916 |
| $L_4$ | 0.734 | 0.880 | 0.871 |
| $L_5$ | 0.734 | 0.813 | 0.848 |
| $L_6$ | 0.731 | 0.879 | 0.815 |

## Results and Discussion

Results of three years according to the morphine percentage in the samples are presented in Table 4.

The most important and potent alkaloid is morphine which can be used for both short term as well as long term pain control, is widely used in many prescriptions of pain medications. The drug occurs as a white crystalline powder or colorless crystals and is available for legal medical use.

Diversity based on alkaloid spectrum in 122 accessions of indigenous opium poppy was undertaken by Shukla *et al.* (2010). They obtained 11 clusters based on extent of correlation between five major alkaloids i.e. morphine, codeine, thebaine, narcotine and papaverine. Mostly the clusters comprised of accessions with different possible combinations of alkaloids comprising high in one alkaloid with high or low of another. Generally the percentage of morphine content was higher than the sum of four other alkaloids except in one cluster where narcotine content was slightly higher than morphine. Dittbrenner *et al*. (2009) evaluated 300 accessions of opium poppy for 35 morphological and agronomic traits collected from all over the world at IPK Gene Bank, Gatersleben, Germany. Based on their study on five major alkaloids taken for two years, they concluded highly significant correlation between total alkaloid content and morphine [18]. Ozturk and Gunlu (2008) conducted correlation and path coefficient analysis for qualitative and quantitative traits in four poppy

cultivars in Central Anatolia. They found statistically significant differences for all the studied traits among all the four genotypes. Positive and significant correlation of morphine yield with morphine content, seed yield, capsule yield, oil yield were noticed [19]. Matyasova *et al*. (2011) evaluated 57 cultivars of opium poppy comparing the groups of values representing the indicators of production-significant morphologic and agricultural traits and morphine content in husk in relation to ideotype, which in these indicators represents 100% of the value. They observed lower values of morphine in husk of white coloured seeds while high morphine in blue to grey seeds. They observed that these cultivars achieved very good values in the morphological indicators and average value in the economic indicators. Based on their results they concluded that these results will be used in selection and classification of suitable genetic resources of poppy as industrial forms [20]. Mishra *et al*. (2010) evaluated progenies of randomly selected individuals from 14 promising hybrids over F2 to F6 generations for opium and seed yield and their contributing traits for the formulation of effective selection strategy in opium poppy (*P. somniferum*).They observed that in general heritability and genetic gain declined from generation to generation [21]. Yadav *et al*. (2009b) examined combining ability for yield and its component traits along with morphine content to elucidate the inheritance pattern governing these traits and also to identify potential genotypes which could be further exploited in breeding programmes. They noticed that most of the traits were governed by non-additive gene action while additive gene action was also important for some other traits [22]. Yadav *et al*. (2009a) analyzed F1 and F2 generations of a twenty parents fractional diallel cross in opium poppy (*P. somniferum*) to estimate the combining ability of the crosses based on ten quantitative and five qualitative (alkaloids) traits. The results indicated that significant differences exist among the parents for all the traits [23]. Based on distinctness in morphological and agronomical characteristics, 1,000 distinct poppy germplasm lines were provided by Agriculture faculty, Ankara University from which 99 poppy lines were evaluated in terms of alkaloid analysis [4]. They observed the range of morphine from 0.110 to 1.140%. Our results were similar to high levels of this trial. Bernath *et al*. (1988), examined the effects of light on *P. Somniferum* within sample cultivars collected from England, Hungary, Afghanistan, India and Thailand, and reported that the morphine rate ranged from 0.28 to 0.38%, 0.66 to 0.75%, 0.26 to 0.56%, 0.24 to 0.58% and 0.17 to 0.35%, respectively [24]. According to our results in the first year, morphine ratios were closed with Hungary cultivar morphine ratios; whereas after selection the ratios of morphine in different lines were increased in the second and Third years. Kapoor (1995), reported that ratio of morphine ranged from 0.22-0.55% in registered native opium poppy cultivars [2]. The results of this trial were less than our results each year.

## Conclusions

In conclusion, selected plants among these six Turkish opium poppy lines based on interested morphine content could be used in breeding programs and could serve as major source of morphine in future. As seen in Table 4. the rate of morphine was low in first year whereas followed by in second and third years after Selection hairless stem and strong capsule types among different lines, morphine percentage lifted in each line. Poppy capsules can be efficiently utilized provided a factory can extract morphine. The importance of the subject can be more comprehended as the loss was considered.

Legal opium poppy production is allowed under the rules of the United Nations and Turkey is one of legal producer. Protection of traditional opium poppy producing position for Turkey is very important. Industrial evaluation of opium poppy and producing high morphine cultivars can protect this position of Turkey.

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