

**MORPHOLOGICAL DIVERSITY OF SOME OPIUM POPPY GENOTYPES
(PAPAVER SOMNIFERUM L.)**

Mirjana JANKULOVSKA^{1*}, Sonja IVANOVSKA¹, Gjoshe STEFKOV², Jelena
ACEVSKA², Dane BOSHEV¹, Ljupcho JANKULOSKI¹

¹Faculty of Agricultural Sciences and Food, UKIM, Skopje, Republic of Macedonia

²Faculty of Pharmacy, UKIM, Skopje, Republic of Macedonia

*(corresponding author: mirjanajankulovska@yahoo.com)

Abstract

Cluster analysis using qualitative variables is a useful tool in estimating genetic diversity between genotypes in a germplasm collection. The objective of this study was to classify opium poppy genotypes based on several morpho-qualitative traits of the flowers, capsules and seed. The collection of 50 poppy genotypes with different origin was evaluated in 2010, on experimental field near Skopje. The classification of the genotypes was done based on Gower distance and the dendrogram was constructed using UPGMA method. Two main clusters were identified, each comprising different number of subgroups. All genotypes with white petal color were grouped in the second cluster. Genotypes with colored petals belonged to the first cluster. The origin of the genotypes had no influence on the classification. The results of this study enabled clear overview of the morphological diversity identified in the studied germplasm.

Key words: opium poppy, morphological diversity, qualitative traits, cluster analysis

Introduction

Republic of Macedonia has a very long tradition in growing opium poppy. In the nineteenth century it was one of the most important crops in Macedonia (Nikolic, 1954) and was used for opium production. Macedonian opium had the highest morphine content in the world (Bensussan, 1946), which, calculated in dehydrated opium varies between 15.9% and 17.2% (Vajic and Mikic, 1951). Today, the opium poppy is grown for its seed and capsules and morphine is extracted from the dry capsules. The production is mainly based on local landraces and one commercial cultivar and as a result, in the recent years, the need to improve existing germplasm became apparent. The renewal and upgrade of the poppy breeding program is of utmost importance.

The main goal in European poppy breeding programs in the long run was to create universal type, that will have high seed yield, suitable color (usually blue) as well as high morphine content in ripe capsule wall, which was in line with the poppy ideotype (Matyasova et al., 2011). One of the most important steps for genetic improvement of crops through conventional breeding is to study the genetic diversity available in the introduced plant/crop material (Mishra et al., 2013). To conduct any breeding program judiciously, diversity analysis based on morphological and biochemical traits is prerequisite. In opium poppy, several collections at different research institutes have been evaluated for genetic diversity. Singh et al. (2004) studied genetic divergence between 101 germplasm lines from different ecogeographical origin based on quantitative traits using multivariate and canonical analysis. They identified clusters with genotypes which had greater potential as breeding material

because they comprised high mean values for one or more component characters and high statistical distances among them. Yadav et al. (2007, a) investigated the genetic divergence in a genetically distinct opium poppy genotypes by cluster and principle component analysis. They recommended some accessions which may be used in hybridization programme to obtain desirable progeny. Yadav et al. (2007, b) also assessed the genetic divergence in a population of 20 parents and 90 F₁ hybrids. All genotypes were grouped into 14 clusters which indicate significant diversity among parents. As a result, considerable variation existed in their crosses. Furthermore, Brezinova et al. (2009) evaluated poppy genotypes from the world collection and observed important diversity for morphological characteristics. Several researchers evaluated the genetic diversity based on alkaloid spectrum (Shukla et al., 2010; Nemeth-Zambori et al., 2011; Stranska et al., 2013).

For establishing an effective breeding program, the genotypes in the starting collection should be grouped according to their characteristics. Therefore, the present study was undertaken with the following objectives: (a) to analyze the genetic diversity among poppy landraces and breeding lines from Macedonia and introduced poppy genotypes based on various morphological traits and (b) to determine the extent of genetic diversity for proper utilization in the breeding program.

Material and methods

A total of 50 opium poppy genotypes were evaluated in randomized block design with 2 replications during 2010-2011. The experimental field was located near Skopje, Republic of Macedonia, between 42°05'N latitude and 21°23' E longitude, 402 m above sea level. The germplasm collection comprised Macedonian land races and advanced breeding lines as well as genotypes of different geographic origin (Table 1). Each plot consisted of two rows, 2 m long, with of 30 cm between rows and 5 cm within row spacing. During the vegetative growth standard agro-technical practices were applied.

The morphological traits were described according to UPOV descriptor (1999). Ten randomly selected plants per replication were marked before flowering and the observations were recorded on 14 morphological characters of the stem (hairiness between the capsule and upper stem leaf), petal (petal color, petal color intensity, presence of blotch, color of blotch, intensity of violet color of blotch), capsule (shape of base, shape of longitudinal section, depth of ribbing and dehiscence, stigmatic disk shape, surface of stigmatic disc lobes, apex of stigmatic disc lobes) and seed (color). The genotypes were clustered based on Gower's distance and the dendrogram was constructed using UPGMA method. The package "cluster" from R statistical software was used for the analyses.

Table 1. Origin and code of poppy genotypes

Origin	Genotype code
Macedonia	29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50
Turkey	12, 13, 20, 21, 22, 23, 24, 25, 26, 27, 28
Bulgaria	4, 5, 6, 7
Australia	1
Austria	2
Belgium	3
Morocco	8, 9, 10
Portugal	11
Ukraine	14
Hungary	15, 16, 17
Sweden	18
unknown	19

Results and discussion

The poppy genotypes expressed various morphological characteristics and attempts have been made to classify them on the basis of qualitative traits. The stems of the poppy plant may be glabrous or hairy. Out of 50 genotypes, 36 had hairs on the stem, between the capsule and the upper stem leaf. The petal color in poppy can vary from white to dark violet, with different intensity. White petal color without blotch was observed in 15 genotypes. 2 genotypes had red petal color with dark violet blotch. The remaining 33 genotypes had different intensity of violet color of the petal and blotch. Brezinova et al. (2009) identified genotypes with different intensity of red petal color.

The form of the capsule is one of the most stable characteristics of the poppy. According to the capsules, the poppy genotypes can be classified in two categories: with closed or with open capsules. Both open and closed capsules can be found on the same plant. The closed capsules do not open automatically when the plant ripens and they have to be thrashed in order to collect the seeds. This trait is desirable in poppy because there is no seed loss due to capsule dehiscence. In this category belonged 42 of the evaluated genotypes, while only 8 had open capsules.

The shape of the capsule is a typical cultivar trait (Brezinova et al., 2009), but it is very unbalanced in genotypes with higher number of capsules that gradually mature. According to Borecki and Stiffel (1995), the breeding aim is a opium poppy cultivar with capsules of globular shape which contains the biggest share of big seeds. Conical shape was detected in 27 genotypes, 10 genotypes had circular capsules and the others had flattened (5), rectangular (5) and elliptic (3) capsules. The colour of the poppy seeds has many variations, but in any one capsule is almost always uniform, only the shade sometimes varies. If there is no cross-pollination, the seed colour is hereditary and remains unchanged. It wasn't possible to establish final correlation between the petal color and seed color, but in most cases the poppy with white or rose-colored flowers have white or light-colored seeds and poppies with violet or dark-colored flowers give blue or dark-colored seeds. Most of the genotypes in this study had different shade of gray color (29), 9 genotypes had white seeds, 5 had ochre color, 8 were with brown seed color and only 1 genotype had pink seed.

Based on the UPGMA method, the germplasm lines were grouped into two main clusters (Figure 1). The cluster I consisted of poppy genotypes with white flowers, while in cluster II

belonged genotypes with violet and red flowers. Each cluster comprised 2 subclusters and different number of genotypes. Cluster I consisted of 15 genotypes that were separated in two main subgroups. In the first subgroup only two genotypes were positioned (16 and 18). The second subgroup comprised 13 non-dehiscent genotypes. Except genotype 25 all other had oval lobus apexes and had white, ochra and brown seeds. For the other characters, all forms were present. The first subcluster from the cluster II contained 6 genotypes. All of them had light or medium intensity of violet color, except genotype 11 with red petal color. All genotypes in this subcluster had flat capsule base and the circular form prevailed. Only genotype 13 had rectangular capsule. In the second subcluster from the cluster II belonged genotypes with light, medium or dark violet color. Genotype 50 had red petal color. Most of the genotypes in this group had flat capsule base, capsules with shallow or medium ribbing, and all forms of capsules except flattened were present. Only three genotypes were dehiscent. The majority had conical capsules and gray seeds. Classification of germplasm lines into different groups or subgroups was independently of their origin. It was difficult to establish any relationship between the origin and clustering pattern.

The preliminary characterization of opium poppy genotypes gives a clear overview of the extend of morphological variability in the existing collection. The genotypes belonging to a particular cluster and having desirable traits can be hybridized with the other promising lines from different clusters, which may facilitate the accumulation of favorable genes in the progeny. The obtained hybrids may be fixed in the advanced generations, which may lead to development of high yielding varieties with desirable characteristics.

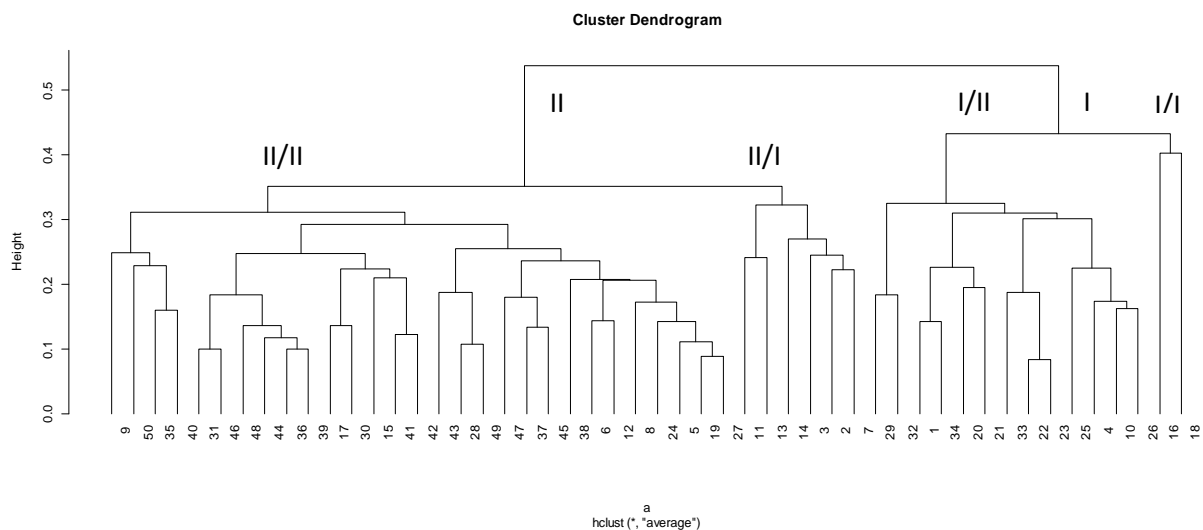


Figure 1. Dendrogram representing genetic distance between 50 opium poppy genotypes.

Conclusions

The analyzed opium poppy genotypes showed high diversity for the morphological traits. Based on the morphological characteristics the germplasm lines were classified in two main clusters. Genotypes that had white petal color without blotch were located in cluster I and the genotypes with colored petals and different intensity of the violet color of blotch belonged to cluster II. All variants of other traits were present in both clusters. There was no association between genotype origin and the clustering pattern. The results of this study were useful for identification of the existing morphological variability in poppy collection. Further research is needed in order to evaluate different quantitative and qualitative traits and perform more precise classification.

References

- Bensussan I. (1946). L'Opium. Pub. Vigot Frcres, Paris, p. 160.
- Borecky V., Stiffel R. (1995). Olejniny (Repka olejná, slne nica ro ná, mak siaty). Nitra, Agroservis Utvip, pp.85-125.
- Brezinova B., Macak M., Eftimova J. (2009). The morphological diversity of selected traits of world collection of poppy genotypes (Genus *Papaver*). *Journal of Central European Agriculture*, 10(2): 183–190.
- Matyasova E., Novak J., Stranska I., Hejtmankova A., Skalicky M., Hejtmankova K., Hejnak V. (2011). Production of morphine and variability of significant characters of *Papaver somniferum* L. *Plant Soil Environ.* 57 (9): 423–428.
- Mishra B.K., Rastogi A., Siddiqui A., Srivastava M., Verma N., Pandey R., Sharma N.C., Shukla S. (2013). Opium Poppy: Genetic Upgradation Through Intervention of Plant Breeding Techniques in Plant Breeding from Laboratories to Fields, edited by S.B. Anderson, InTech, 288 p.
- Nemeth-Zambori, E., Jaszberenyi, C., Rajhart, P., Bernath, J. (2011). Evaluation of alkaloid profiles in hybrid generations of different poppy (*Papaver somniferum* L.) genotypes. *Industrial Crops and Products* 33: 690–696.
- Nikolic D. (1954). Problem opiuma u svetu i kod nas, Nova trgovina, 10, p. 592
- R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org/>.
- Shukla S., Yadav H.K., Rastogi A., Mishra B.K., Singh S.P. (2010). Alkaloid diversity in relation to breeding for specific alkaloids in opium poppy (*Papaver somniferum* L.). *Czech Journal of Genetics and Plant Breeding*, 46(4): 164-169
- Singh S.P., Shukla S., Yadav H.K. (2004). Multivariate analysis in relation to breeding system in opium poppy (*Papaver somniferum* L.). *Genetika*, 36: 111-120.
- Stranska I., Skalicky M., Novak J., Matyasova E., Hejnak V. (2013). Analysis of selected poppy (*Papaver somniferum* L.) cultivars: Pharmaceutically important alkaloids. *Industrial Crops and Products*, 41: 120– 126.
- UPOV, Guidelines for the conduct of tests for distinctness, uniformity and stability, Opium/Seed poppy Geneva (*Papaver somniferum* L). UPOV, Geneva, 1999.
- Vajic B., Mikic F. (1951). O sadrzaju vode i morfina u jugoslavenskom opiumu. Special edition of the Serbian Academy of Sciences, Belgrade, p. 5.
- Yadav H.K., Shukla S., Rastogi A., Singh S.P. (2007a). Assessment of diversity in new genetic stock of opium poppy (*Papaver somniferum* L.). *Indian Journal of Agricultural Sciences*, 77(8): 537–539.
- Yadav H.K., Shukla S., Singh S.P. (2007b). Genetic divergence in parental genotypes and its relation with heterosis, F1 performance and general combining ability (GCA) in opium poppy (*Papaver somniferum* L.). *Euphytica*, 157: 123–130.