Journal of Medicinal Plants and By-products (2024) 1: 113-121
https://doi.org/10.22034/jmpb.2023.361063.1521

Original Article

Growing, Flowering and Seeding characteristics of Two Forest Violas (*Viola caspia* and *Viola sintenisii*) Compared to *Viola tricolor* under Controlled Conditions

Ali Ammarellou[1*] and Valiallah Mozaffarian[2]

[1]*Research Institute of Modern Biological Techniques, University of Zanjan, Zanjan, Iran.*
[2]*Research Institute of Forests and Rangelands, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran.*

Article History

Received: 01 January 2023
Accepted: 20 February 2023

Keywords
Forests
Viola Species
Diversity
Flowering
Seed Sitting

***Corresponding author**
Amarlou@znu.ac.ir

ABSTRACT

Forests, along with their great advantages in environmental sustainability, are suitable and exclusive habitats for important plant species, including violets. Viola is an important forest plant in terms of recognizing flowering systems diversities, ornamental and medicinal importance. Medicinal violets are generally wild and natural. Cultivation and industrialization of drug production process from these medicinal plants, it is necessary to review and re-research them in controlled conditions. In this study, morphological differences, plant growth and developmental behavior especially flower buds production were studied in three different species of Viola: *Viola caspia* (Rupr.) Freyn, *V. sintenisii* W.Becker and *V. tricolor* L. (under greenhouse and field conditions). All greenhouse and field experiments were performed in a completely randomized design (CRD) and randomized complete block design (RCBD) with three replications, respectively. The species with a purple flowers (*V. sintenisii*) blooms about fifteen days earlier than a white flower violet (*V. caspia*) in spring season. Compared to these two species, the *tricolor* species blossoms two months earlier. Appearance and morphological tolerance to winter cold was the highest in *V. tricolor* and then in *V. sintenisii* was second. The *V. caspia* tolerance to winter cold was lowest. Flower buds in the blue species (*V. sintenisii*) are formed in the first node located on the meristem and form a flower shoot up to a height of 11 cm. In white flowers (*V. caspia*) and ornamental violet (*V. tricolor*), unlike the first species, flower buds form on higher internodes, which are about 5 cm higher than the original node. The color of the underground stem in *V. sintenisii* and *V. caspia* was white and brown respectively. The type of flower produced in *V. sintenisii* and *V. tricolor* were chasmogam at all blooming time, but in the *V. caspia* it is of the type of cleistogam, semi-chasmogam and chasmogam. The size and shape of the fruits formed on all three studied species are different, which is round in *V. sintenisii* and elliptical in *V. caspia* and *V. tricolor*. The *V. sintenisii* grow in a sleeping and rosette state and tend to hang in pots, however the *V. caspia* and *V. tricolor* are completely vertical and non - rosette. The amount of violet scent (olfactory test) was the highest in *V. sintenisii* and then second in *V. caspia* but ornamental violet (*V. tricolor*) had no odor. Because the most of medicinal spices of viola are wild, it is challenging for compare of their different species for morphological and physiological characteristics as well as produce a standardized product with a high content of specialized metabolites (SM). To overcome this great challenge, this study focused on the evaluation of growing, flowering and seeding characteristics of 3 forest violas under controlled conditions. This information will be so important for their domestication, cultivation, mass propagation and plant breeding programs.

INTRODUCTION

The Violaceae comprises 500-600 predominately tropical and temperate species in 25 currently recognized genera. Viola section is one of the largest groups of the Violaceae family. Infra generic classification has varied, but recent phylogenetic analysis indicates that the genus can be subdivided into two subgenera and 16 sections worldwide [1-4]. Some *Viola* species are perennial plants, some are annual plants, and a few are small shrubs. Many species, varieties and cultivars are grown in gardens for their ornamental flowers. In horticulture the term pansy is normally used for those multi-colored, large-flowered cultivars which

are raised annually or biennially from seed and used extensively in bedding. The terms viola and violet are normally reserved for small-flowered annuals or perennials, including the wild species. Green space violet is one of the cold-resistant ornamental plants whose seedlings are planted in early autumn in cold regions. These plants reach the flowering stage in mid-winter to late spring and gradually seeded and die as the weather warms in annual species. Around 30 species have been identified in north and northwest of Iran of which 19 species are native to Iran. The most important medicinal species of viola genus are *V. arvensis*, *V. baoshanensis*, *V. odorata*, *V. caspia* and *V. sintenisii* [4-6]. According to published scientific reports, most of the dense habitats of Iranian violets are located in the forest areas of Golestan, Mazandaran and Gilan provinces, which are among the three dominant and hot habitats in Iran [3,4,6]. Green space violet (*V. tricolor*) is one of the cold-resistant ornamental plants whose seedlings are planted in early autumn in cold regions. Despite of all the research that has been done on different species of violets in the world [7,8], there are very few scientific results under uniformly controlled experimental conditions. In addition to medicinal and ornamental importants, the genus of viola is well known for the presence of cleistogamous (CL) flowers and by the singularity of its chasmogamous (CH) flowers, which have attracted the attention of pollination biologists since Sprengel (1793) [9]. Floral biology and pollination mechanisms have been studied in several North American and European species [10-14].

The infrageneric taxonomy for Viola based on modern principles of phylogenetic and monophyly and the accumulated information was generated by Thomas Marcussen in 2022 [15]. According to the routine process and standard flora studies, all reports and sources have been compiled and references of published plant flora are based on collected and stored dried and fixed samples in national and international herbariums. This valuable work has its limitations as well as its numerous advantages. Samples collected from different climates and geographical areas, despite the apparent differences in their morphology, can be of the same species and the same variety and genotypes. Due to the fact that most of the identifications and reports of herbarium are based on morphological studies and their physical characteristics and are less identified and classified based on molecular information, so the probability of error in diagnosis will be high. On the other hand, dry specimens of leaves, stems and flowers cannot be regenerated, growth and reproduction, and their seeds lose their ability to grow and germinate after a limited time. For these reasons, the creation of Live Plant Collections (LPC) have the more particular importance. In these collections, the collected plant specimens, whether as whole live plants or as seeds, are continuously grown and kept alive in greenhouse and field conditions. Under these conditions, climatic and environmental differences will be minimized and the detection of samples will be very accurate. Simultaneous and comparative studies between different masses will also be possible. In these collections, researchers can access samples and fresh plant tissues in all seasons and any research can be done on them in terms of techniques and statistical requirements and experimental design. As mentioned, the medicinal violets are generally wild and natural, and there are few studies on them under the same environmental conditions. It is obvious that different climates as well as soil and habitat conditions have different effects on growth characteristics as well as medicinal properties of wild species that should be cultivated in uniform environmental conditions to study the apparent diversity of metabolic multiplicity. The review and re-research on medicinal species of viola biological behaviors are necessary for their cultivation programs and industrialization of drug production process.

MATERIALS AND METHODS

Live Collection of Iranian Viola sp. (LCIV)

According to published scientific reports, most of the dense habitats of Iranian violets are located in the forested areas of Golestan, Mazandaran and Gilan provinces, which are among the three dominant and hot habitats in Iran (Fig. 1). Accordingly, most of the samples collected in the first phase of creating a live collection of Iranian viola sp. focused on the three provinces and more than 20 northern ecotypes of medicinal violets are collected and transferred to this collection.

This national live collection of violas (LCV) ecotypes is located in the province of Zanjan (Research Institute of Modern Biological Techniques, University of Zanjan-Iran) at latitude

36°68′ N, longitude 48°38′ E, and an altitude of 1579 m with cold climate, mean annual precipitation of 295 mm, and mean annual temperature of 10 °C (Fig.3-7). The average minimum temperature in the coldest month is " February to -7.5 °C" and the average maximum temperature in the warmest month is "August, 32.1 °C". During the year, the temperature drops below zero for 118 days, with January and February ranking first with 27 days. The wettest month is May with 52.5 mm and the driest month is September with 3.5 mm precipitation. The maximum recorded wind speed of 27 meters per second was "97 kilometers per hour". The prevailing wind in Zanjan in most months of the year was in the east and the average wind speed of 3 meters per second is "11 kilometers per hour".

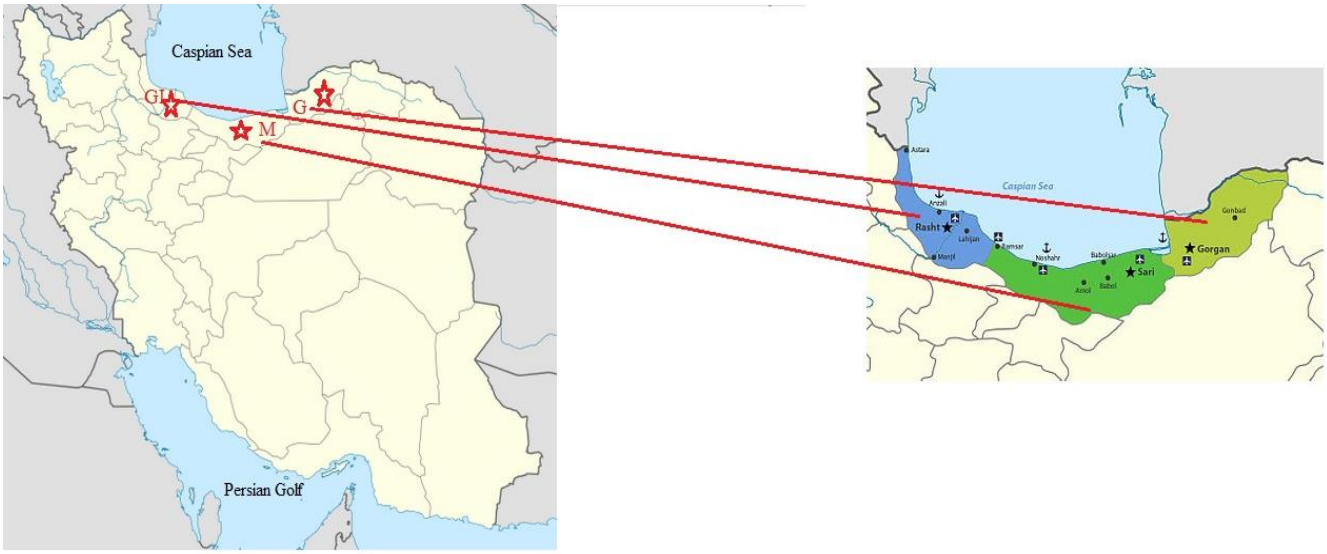

Fig. 1 Three hot point of Viola sp. habitats in Iran. These important wild habitats included: Gilan, Mazandaran, Golestan, Arasbaran and Northern Alborz chain Mountain

Ex Situ and in situ Evaluations

Frequent and regular visits at different stages of violet habitats were performed in the northern provinces of Iran and the necessary morphological studies were evaluated. Appearance characteristics of plants including growth type, height, flowering time, flower type, petal diameter, flower weight, flower color, flower structure and fruit organization, number of meristematic flower buds were analyzed in LCV and the results of observations were recorded in greenhouse as well as in field. The photoperiod conditions of the greenhouse consisted of 10 hours of natural light and 14 hours of darkness with an average temperature of 25 ˚C±4 and 70% humidity.

Statistical Analysis

All greenhouse and field experiments were performed in a completely randomized design (CRD) and randomized complete block design (RCBD) with three replications, respectively. All of the obtained data were analyzed by one-way ANOVA using *SPSS Statistics* for Windows, Version 23.0 (IBM *SPSS Statistics* for Windows, Version 23.0. Armonk, NY: IBM. and significant differences among the treatments were analyzed using Fisher's Least Significant Difference (LSD) at the 0.01 level of significance.

RESULTS

Based on the periodic visits observations in the Caspian forests and parks of northern Iran, especially in the Lahijan forest region, in the spring, two species of medicinal violet begin to grow and flower at the base of trees and even the trunks of forest and parks trees (Fig. 2). Both species are perennial and are used medicinally in local folklore. Based on morphological characteristics, vegetative and growth characteristics including leaf shape, flower color, growth type, fruit shape, etc. these violets were identified as: *V. alba* Bess, subsp. *sintenisii* and *V. caspia.* In addition to the habitat data, the species transferred to the LCV were studied under greenhouse and non-habitat field conditions (Province of Zanjan). All results, observations and studies of different plant species under the same biological conditions (LCV) provide

more documented and valid information (Tab.1 & 2) on plant genetic differences (Fig. 2-16).

Based on continuous morphological studies over more than three years on samples collected from Gilan, Mazandaran and Golestan provinces, two medicinal species of *V. alba* Bess, subsp. *sintenisii* and *V. caspia* are the two dominant species in the mentioned areas (Ammarellou *et al*., 2021), which are shown in the relevant pictures (Fig. 4-14).

Fig. 2 View of the park in Lahijan, Iran (A) and the growth of white violets on the trunk covered with ornamental palm moss (B,C,D,E).

Fig. 3 Local live collection of Iranian Viola sp. Located on Research Institute of Modern Biological Techniques, University of Zanjan, Zanjan, Iran.

Fig.4 Morphological characteristic of *V. alba* subsp. *sintenisii.* established on LCV.

Fig. 5 The seed setting and fruiting of *V. alba* subsp. *sintenisii* in LCIV

Fig. 6 Different stages of fruit and seed maturing in *V. alba* subsp. *sintenisii* in LCIV

Fig. 7 Morphological characteristic and seed setting and fruiting of *V. caspia* in LCIV

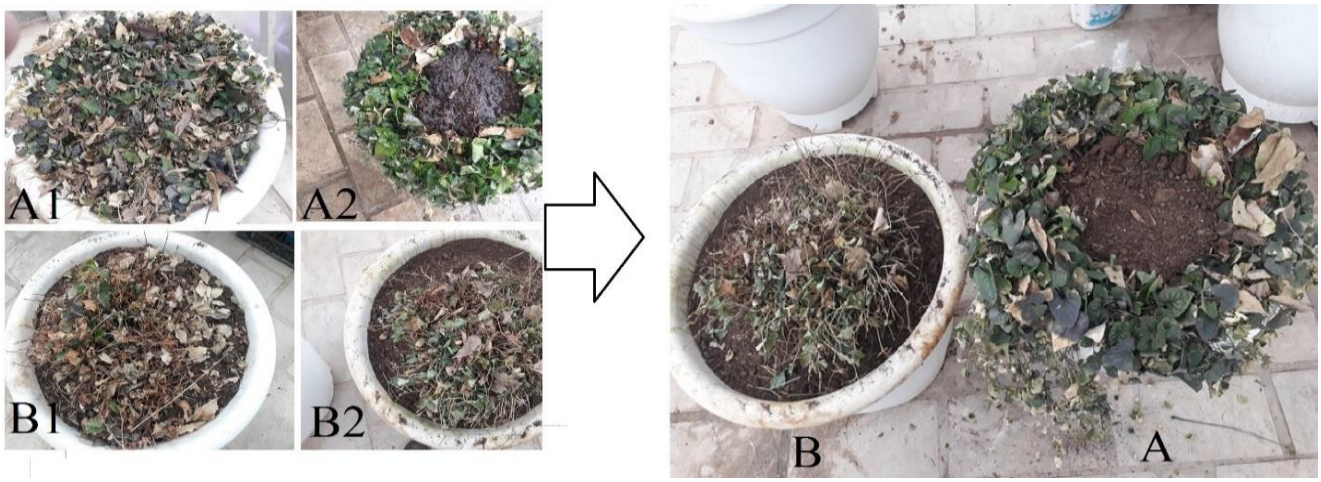

Fig. 8 Different effects of winter cold on the studied species. A1: *V. caspia,* B1: *V. sintenisii* in left and A: *V. sintenisii,* B: *V. caspia.* Tolerance to winter cold in the *V. sintenisii* is more than the *V. caspia*.

Fig. 9 Peak and maximum flowering in *V. sintenisii*

Fig. 10 Activation of flower meristem buds in mid-winter on *V. sintenisii*

Fig. 11 Differences in the color and size of the underground stems in the two studied species.

Fig. 12 Origin of flowering shoots in studied species and comparison of peduncle length from origin meristems. A & B: *V. caspia*, C: underground stem of *V. caspia*. D & E :Flower formation in higher plant nodes in the species *V. caspia*.

Fig. 13 Growing view of 2 studied species. A: *V. sintenisii*, B: *V. caspia*

Fig. 14 Initiation of flower-producing meristems in the *V. sintenisii* species under field conditions in accordance with greenhouse observations.

Fig. 15 Initiation of flower-producing meristems in the *V. tricolor* species under field conditions in accordance with greenhouse observations.

Table 1 Flower and leaf characteristics in white chasmogame(WCH), white cleistogame(WCL), Semi white shasmogame(SWCH) and violet chasmogamous(VCH) flowers in 3 studied species.

Flowers type	weight of flowers (g)	weight of peduncle (g)	peduncle long (cm)	leaf weight (g)
WCH (*V. caspia*)	0.061 c	0.095 c	5.8 b	0.42 b
WCL (*V. caspia*)	0.025 d	0.035 d	3.2 d	0.41 b
SWCH (*V. caspia*)	0.024 d	0.034 d	3.1 d	0.40 b
VCH (*V. sintenisii)*	0.095 b	0.155 b	11.8 a	1.5 a
VCH (*V.tricolor*)	0.42 a	0.5 a	4 c	0.23 c

Numbers with common letters in each column do not have significant differences ($P < 0.01$).

Table 2 Mean comparisons of the 3 species of viola for morphological traits

Species	Plant heigh (cm)	Leaf length (cm)	Leaf width (cm)	Root length (cm)	Petiole length (cm)	Leaf number	Pedicle length (cm)	Stem diameter (cm)	Root diameter (cm)
V. sintenisii	11 c	6 a	4a	15 a	8 a	40 a	11.8 a	3 bc	10 a
V. caspia	17 a	4 b	3 ab	13 ab	6 b	30 b	5.8 b	4 b	5 b
V. tricolor	14 b	3 bc	1.5 c	8 c	2 c	10 c	1 c	6 a	3 c

Numbers with common letters in each column do not have significant differences ($P < 0.01$).

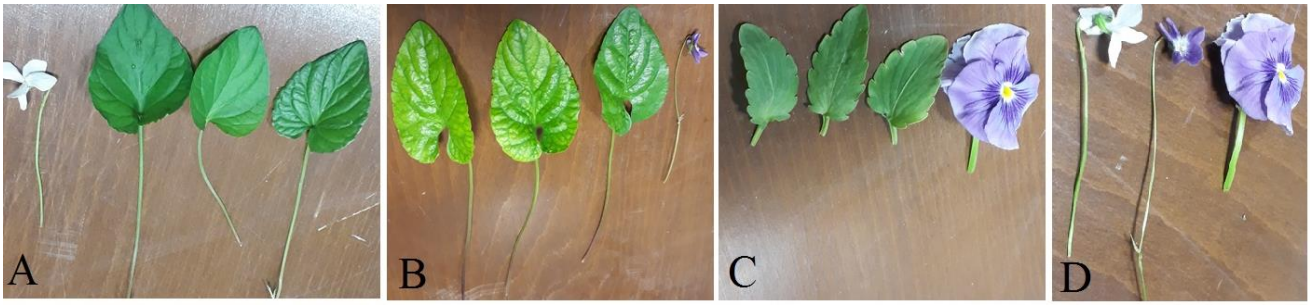

Fig. 16 Comparison of flower types, leaf shape and plant petiole characteristics in *V. caspia* (A), *V. sintenisii* (B) and *V. tricolor* (C). Three chasmogamous flowers in 3 studied species (D).

According to presented results, the species with a purple flowers (*V. sintenisii*) blooms about fifteen days earlier than a white flower violet (*V. caspia*) in spring season. Compared to these two species, the *tricolor* species blossoms two months earlier. Appearance and morphological tolerance to winter cold was the highest in *V. tricolor* and then in *V. sintenisii* was second. The *V. caspia* tolerance to winter cold was lowest. Flower buds in the blue species (*V. sintenisii*) are formed in the first node located on the meristem and form a flower shoot up to a height of 11 cm. In white flowers (*V. caspia*) and ornamental violet (*V. tricolor*), unlike the first species, flower buds form on higher internodes, which are about 5 cm higher than the original node. The color of the underground stem in *V. sintenisii* and *V. caspia* was white and brown respectively. The type of flower produced in *V. sintenisii* and *V. tricolor* were chasmogam at all blooming time, but in the *V. caspia* it is of the type of cleistogam, semi-chasmogam and chasmogam (Tab. 1). The size and shape of the fruits formed on all three studied species are different, which is round in *V. sintenisii* and elliptical in *V. caspia* and *V. tricolor*. The *V. sintenisii* grow in a sleeping and rosette state and tend to hang in pots, however the *V. caspia* and *V. tricolor* are completely vertical and non - rosette. The amount of violet scent (olfactory test) was the highest in *V. sintenisii* and then second in *V. caspia* but ornamental violet (*V. tricolor*) had no odor. Because the most of medicinal spices of viola are wild, it is challenging for compare of their different species for morphological and physiological characteristics as well as produce a standardized product with a high content of specialized metabolites (SM). To overcome this great challenge, this study focused on the evaluation of growing, flowering and seeding characteristics of 3 forest violas under controlled conditions. This information will be so important for their domestication, cultivation, mass propagation and plant breeding programs.

Considering that the two studied medicinal species of Viola (Banafshe -Persian), for the first time have been live collected and studied under domesticated and control conditions, so the detailed report of the growth and development habits of these two important medicinal species with compared to the ornamental species are reported in the first time.

REFERENCES

1. Ballard H.E., Sytsma K.J., Kowal R.R. 1999. Shrinking the violets: phylogenetic relationships of infrageneric groups in Viola (*Violaceae*) based on internal transcribed spacer DNA sequences. Syst Bot. 23: 439-458.
2. Yockteng R., Ballard H.E., Mansion G., Dajoz I., Nadot S. 2003. Relationships among pansies (*Viola* section Melanium) investigated using ITS and ISSR markers. Plant Sys Evo. 241: 153-170.
3. Abolghasemi S., Naderi R., Fattahi Moghadam M.R. 2020. Evaluation of genetic diversity in Iranian Violet (*Viola* spp) populations using morphological and RAPD

molecular markers. J Genet Resour 6(2): 157-171. doi: 10.22080/jgr.2020.18739.1190

4. Ammarellou A., Zabicka J., Słomka A., Bohdanowicz J., Marcussen T., Kuta E. 2021. Seasonal and Simultaneous Cleistogamy in Rostrate Violets (Viola, subsect. Rostratae, Violaceae). Plants, 10, 2147. https://doi.org/10.3390/ plants10102147.
5. Tutin T.G., Heywood V.H., Burges N.A., Valentine D.H., Walters S.M.., Webb D.A. 1964. Flora Europaea. Vol. 1. Lycopodiaceae to Platanaceae. Flora Europaea. Vol. 1. Lycopodiaceae to Platanaceae.
6. Mozafarian V. 1996. A dictionary of Iranian plant names. Tehran: Farhang Moaser, 396.
7. Marcussen T, Borgen L. 2011. Species delimitation in the Ponto-Caucasian *Viola sieheana* complex, based on evidence from allozymes, morphology, ploidy levels, and crossing experiments. *Plant Syst Evol* 291: 183-196.
8. Marcussen T., Nordal I. 1997. *Viola suavis*, a new spice in the Nordic Flora, with analysis of the relation to other species in the subsection Viola (Vioalceae). *Nordic J Bot* 18: 221-237.
9. Sprengel C.K. 1793. Das entdeckte Geheimniss der Natur im Bau und in der Befruchtung der Blumen. Berlin: Vieweg sen. Reprint 1972, Lehre: J Cramer & HK Swann, Codicote, New York: Wheldon & Wesley. [Google Scholar].
10. Freitas L, Sazima M. 2003. Floral biology and pollination mechanisms in two Viola species--from nectar to pollen flowers? Ann Bot. Feb;91(3):311-7. doi: 10.1093/aob/mcg025. PMID: 12547683; PMCID: PMC4244963.
11. Knuth P. 1904. Handbuch der Blütenbiologie, v. III. p. 1. Leipzig: Verlag von Wilhelm Engelmann. [Google Scholar].
12. Beattie AT**.** 1969*a* Studies in the pollination ecology of *Viola* 1. The pollen-content of stigmatic cavities. Watsonia 7: 142-156.
13. Beattie T**.** 1969*b* The floral biology of three species of *Viola* New Phytologist 68: 1187–1201. [Google Scholar]
14. Beattie A.T. 1971. Pollination mechanisms in *Viola* New Phytologist 70: 343–360.
15. Marcussen T., Ballard H.E., Danihelka J., Flores A.R., Nicola M.V., Watson J.M. 2022. A Revised Phylogenetic Classification for *Viola* (Violaceae). Plants. 11, 2224. https://doi.org/10.3390/plants11172224.