

Studies on the propagation and behavior of some biotypes of *Mirabilis jalapa* L.

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ABSTRACT

Nowadays, worldwide, but also in Romania, there is an enormous demand for *Mirabilis jalapa* L., a popular ornamental plant for landscaping design, due to the beauty of its flowers, but also for its use in traditional medicine. This study highlights some contributions regarding generative propagation in *Mirabilis jalapa* L., which is appreciated, especially as a decorative plant, alone or in different combinations, but also for its uses, mainly in medicine. Experience shows that *Mirabilis jalapa* L. can be easily used as a teaching plant in the practical study of plant morphology and physiology, as well as in the demonstration of certain genetic particularities. In this experiment, seeds from 4 biotypes with flowers of different colors (red, pink, white, and yellow) were tested for germination percentage and number of viable plants, under greenhouse conditions. The morphological characteristics of these seeds and the plants obtained from these were also evaluated. The maximum germination percentage was 100% for the red flower variant, followed by the yellow flower variant, with a percentage of 95%. The germination interval is between 6 and 20 days, with a maximum at 15 days after sowing. The most vigorous was the white-flowered variant, which had a maximum height (110.3 cm) and also the largest diameter (60.9 cm). The seeds are relatively large, generally ranging between 6.6 and 8.8 mm in height and 4.0 and 5.7 mm in diameter, respectively.

Keywords: four o'clock flower, annual plant, propagation, morphological characteristics

INTRODUCTION

Mirabilis is a genus of the Nyctaginaceae family, mainly native to America, and commonly known as the four o'clock flower. It is a perennial bushy herb that propagates by flowers or leaves. The genus *Mirabilis* comprises approximately 50 species distributed primarily in tropical and temperate regions of the world, and of these, the most cultivated as an annual plant is *M. jalapa* L.

In Romania, when grown outdoors in the field, it behaves like an annual plant due to the climatic conditions during the winter period (Toma and Petra, 2020). However, due to climate change, it could perhaps be cultivated as a perennial plant, both in the coastal area and in the Danube Delta area, where the cold period is shorter, the minimum temperatures are closer to zero degrees, and the ground frost is superficial. *M. jalapa* L. is native to the dry

tropical regions of Belize, El Salvador, Guatemala, Honduras, Mexico Central, Mexico Gulf, Mexico Northeast, Mexico Northwest, Mexico Southeast, Mexico Southwest, Nicaragua, but it has been naturalized in many parts of the world (Asia, Africa, the United States, the Middle East, Europe, as well as in Australia) (<https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:162591-2>)(Figure 1). It was introduced to Europe in the 16th century, after the Spanish conquest of Mexico, when the seeds of *M. jalapa* reached Spain, then England and then spread throughout the European continent, being cultivated as an ornamental plant (Engels *et al.*, 1974; <https://ro.guide-herb.com/kogda-sejat-mirabilis>). In Romania, *M. jalapa* L. is mainly present in the area of large localities (Alba Iulia, Bucharest, Cluj-Napoca, Craiova, Constanța, Galați, Iași, Pitești, Suceava, Târgu Mureș, Timișoara etc.)(Figure 2).

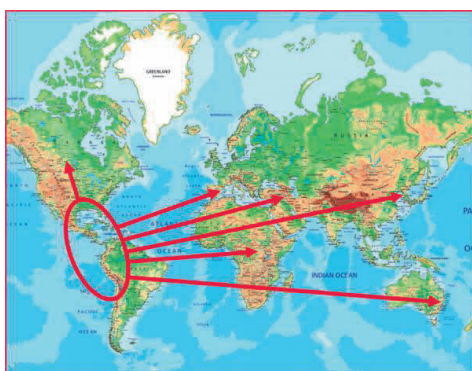


Figure 1. The origin and global spread of *M. jalapa* L.

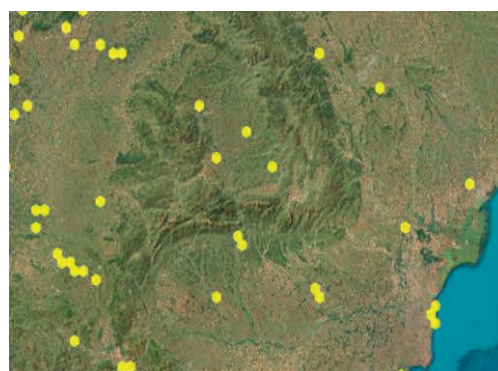


Figure 2. The spread of *M. jalapa* L. in Romania

Source: <https://identify.plantnet.org/ro/the-plant-list/species/Mirabilis%20jalapa%20L./data>

Mirabilis himalaica is indigenous to the Himalayas, being the only species native outside the northern hemisphere, most species of the genus being native to the tropical and subtropical areas of South America (Singh *et al.*, 2012). Some species are ornamental and are cultivated in the world's warmer parts. Several of the American species are widespread.

The flower plant (scientifically known as *Mirabilis jalapa*) is now found in most parts of the world. Its trumpet-shaped blooms often emerge in the late afternoon, around four o'clock, and remain open until the next morning, hence the name "Four O'Clock Flower". The common name of 'four o'clocks' was given to *M. jalapa* because the flowers of this plant do not open until late in the day. In Romanian the common name is 'frumoasa nopții', 'barba împăratului', 'noptițe', and in French 'belle de nuit' (Toma, 2009; Șelaru, 2007).

M. jalapa L. was used for health care and medicinal purposes for several thousand years. The root was used by indigenous peoples for medicinal purposes, as a hallucinogen, and as a purported aphrodisiac, while the flowers produce an edible red dye for coloring food (<https://hort.extension.wisc.edu/articles/four-oclocks-mirabilis-jalapa>).

This plant was widely used as traditional, herbal based natural therapies from ancient times in different countries. It is now a widely available weed cultivated for medicinal and ornamental purposes (Liyaet *al.*, 2021).

Numerous phytochemical products, including alkaloids, brassicasterol, carbohydrates, flavonoids, glycosides, phytosterols (beta-sitosterol and stigmasterol), oleanolic acid, trigonelline, and ursolic acid, have been identified in this plant's constituent parts.

The anti-inflammatory, antimicrobial (antiviral, antibacterial, and antifungal), antioxidant, cytotoxic, antitumor, antinociceptive, analgesic, antihistamine, immunomodulatory, anti-tubercular induced hepatotoxic effect, antiparasitic, dermatological, hypoglycemic, and antihyperlipidemic qualities of this plant have all been investigated. In the process of

creating gold nanoparticles, it is also employed as a reductant and has many other pharmacological effects (Raza *et al.*, 2024; Al-Snafi *et al.*, 2021; Disha *et al.*, 2024; Peiris *et al.*, 2022; Ramesh and Mahalakshmi, 2014; Saha *et al.*, 2020). It has traditionally been used in the treatment of gastrointestinal disorders, muscle pain, abdominal colic, and diarrhea in ancient Mexico, Japan, China, and Brazil. In the aerial parts of the *M. jalapa*, triterpenes and flavonoids are found. Flowers mostly contain anthocyanins and flavonoids. Carbohydrates, resin, and alkaloids are found in roots. Tricosan-12-one, n-hexacosanal, β -sitosterol, and tetracosanoic acid were isolated from the leaves of the *M. jalapa*. Seeds contain β -sitosterol, β -amyirin, and β -sitosterol-D-glucoside. The presence of various bioactive compounds validates the whole plant for different medicinal practitioners (Kaladhar, 2010). It has been extensively used in almost all folk remedies worldwide for treating a variety of conditions. It has also been used as reducing agent for the production of gold nanoparticles (Liya *et al.*, 2021). *Mirabilis* plants contain antimicrobial peptides, with the potential for their use in the preparation of biopesticides (Maulina *et al.*, 2018). Edible crimson dye from flowers; is used as a colorant for cosmetics, cakes, and jellies. Pounded seeds are used in Malaya, China, and Japan for making cosmetic powder. Powdered root, is also used for cosmetic preparations, mixed with rice powder and sandalwood. In China, the flowers are also used for cosmetic purposes (Campbell *et al.*, 2019; <https://www.stuartxchange.com/AlasCuatro>). As an ornamental plant, *M. jalapa* L. has gained significant attention for its remarkable ability to hyper-accumulate heavy metals such as Cd, Sn, Cu, Pb, and Zn (Li *et al.*, 2020a; Li *et al.*, 2020b; Sun *et al.*, 2011; Li *et al.*, 2025). Beyond its capacity for metal uptake, *M. jalapa* demonstrates tolerance and even degradation capabilities for organic pollutants, including petroleum hydrocarbons and nitrobenzene (Zhou *et al.*, 2012). The use of ornamental plants for phytoremediation of polluted soil can not only remove pollutants from the environment, but also green, beautify, and purify the environment (Li *et al.*, 2020; Li and Xu, 2022).

M. jalapa is also known as a model organism in botanical and genetic studies (Van Dijk and Elis, 2022; Zhang *et al.*, 2022).

M. jalapa L. is right at home in a cottage garden, in borders and beds, or can be added to large containers, impressing with the multitude of color combinations it offers. If possible, place them along lighted walkways where the night-blooming flowers and fragrance can be appreciated. It combines nicely with Asiatic lilies or other plants, creating an interesting contrast between large and small flowers (<https://hort.extension.wisc.edu/articles/four-oclocks-mirabilis-jalapa/>).

In Romania, it is not very widespread, but it is cultivated as an ornamental plant. Dwarf varieties are suitable for landscaping plant bands, flower beds, rondos, and borders, while tall varieties can be used for solitary use, in groups next to shrubs and subshrubs, and in mixed borders (Toma and Petra, 2020). It is not resistant to cold, therefore during the cold season it is recommended to grow it under glass (Mc Hoy, 1997). The ease of propagation by generative or vegetative methods, the low requirements for environmental factors, and the resistance to diseases and pests make *M. jalapa* L. a species recommended to be used as a nocturnal alternative in parks, plant bands, curbs, borders, or even on terraces in pots or planters. They emit a sweet-smelling fragrance throughout the night, and then close in the morning.

M. jalapa is easiest to grow from seed. The seeds are large (caper-sized) and can be sown singly in pots or cells, in warmth, in spring. The seedlings are large and easy to look after if protected from frost until planting out in late May in Romania.

Planted in good soil in full sun, they quickly make large, bushy plants, 60-90cm high and wide. The foliage is narrowly heart-shaped, slightly cupped, and rich green, and the flowers appear at the ends of the shoots in small clusters. The colour of the flowers varies from white to yellow, pink, red, and orange, but what makes the plant remarkable is that two blooms on the same stem can be different colours. In fact, many seed strains offer plants

with flowers that are striped with two contrasting colours or solid-coloured flowers of two hues on the same plant (<https://thebikinggardener.com/2014/08/14/something-truly-wonderful-mirabilis/>). An interesting feature of *M. jalapa* is that the same plant can have flowers of different colors, or individual flowers with color combinations in the form of stripes, spots, sections. In addition, the phenomenon of color change as the plant reaches maturity (for example, from yellow to dark pink, or from white to light purple) frequently occurs (Engels *et al.*, 1974; Rozina, 2016).

In recent years, there has been a great deal of interest in propagating *M. jalapa* plants through tissue culture (Gaber, 2015; Rahman *et al.*, 2021).

This study places particular emphasis on the propagation of the *M. jalapa* species, which could be introduced into landscaping and floriculture on a larger scale in our country.

MATERIALS AND METHODS

The experiments were performed at the Horticultural Research Station-University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca and consisted of testing the possibilities of propagating some biotypes of *M. jalapa* through the generative method and its influence on plant growth and development. Fruits of *M. jalapa* are coriaceous obovoid, called achenes, which become wrinkled and dark brown to black at maturity, and tuberous stems are prominent (Argade and Solanki, 2021).

Seed is covered with a thin fruit wall, tight-fitting, spheroid, 4–6 mm (<http://w3.biosci.utexas.edu/prc/K12/pages/Mirabilis>).

The biological material consisted of seeds purchased commercially. The experiment was monofactorial organized with seeds from four biotypes with flowers of different colors (V₁- red flower; V₂- pink flower; V₃- white flower; V₄- yellow flower), arranged in four repetitions, using 40 seeds of each cultivar.

For sowing or planting tubers, commercially purchased substrate mixed with perlite in a ratio of 10:1 was used, and the soil pH was permanently maintained at 6.8. The temperature inside remained around 21-22°C during the day, dropping to 4-5°C at night, and the relative humidity was 55%.

The seeds were sown in plastic pots, in the greenhouse the end of February 2023, and during the summer the fruits were harvested for re-sowing the following year. In the fall, after the plants died, the tuberous roots formed during the almost eight months of vegetation were harvested. The seeds were kept in paper envelopes at room temperature and the tubers in a pot, covered with soil, at positive temperatures and were sprayed with water once a month to avoid dehydration.

Before sowing, 20 seeds were prepared from each basic biotype (red, white, yellow, and pink), to be analyzed. Germination of seeds was determined. Data on different germination parameters were recorded after germination at 6-day intervals until no further germination occurred. Final germination percentage (%), seedling length, was recorded after 20 days of planting. The germinated seeds were counted daily for 20 days. Seedlings were considered to have emerged when cotyledons were visible at the soil surface. Specific observations of four o'clock plant genotypes were made on the obtained plants regarding morphological biometric data that targeted plant height and diameter, number of flowers per plant, and flower diameter.

Statistical analysis

All the experiments were carried out in single row block method with four replications. Experimental data were analyzed using Microsoft Office-Excel and by a statistical package ANOVA, mean values were considered significantly different if the calculated P value was at least 5% ($P < 0.05$) (Ardelean *et al.*, 2002).

RESULTS AND DISUSSIONS

Results of generative propagation

Seed propagation of *M. jalapa* has a greater share in Romania, but has not been studied to date, and propagation by tuber roots is almost unknown and practiced only by amateur gardeners. For the morphological analysis of the seeds obtained, 20 seeds were randomly selected from each experimental variant and measurements were made regarding their diameter and height (Table 1).

Table 1. Morphological characteristics of the seeds of *M. jalapa* L.

No. crt.	Flower color of the variant							
	Red		Pink		White		Yellow	
	H (mm)	D (mm)	H (mm)	D (mm)	H (mm)	D (mm)	H (mm)	D (mm)
1	8.0	4.7	8.1	4.9	7.8	4.9	7.7	5.4
2	7.1	4.4	7.4	4.2	8.2	5.2	7.7	5.7
3	7.0	4.2	7.9	5.1	8.1	4.6	7.8	4.8
4	7.4	4.7	8.0	5.0	7.8	4.5	7.8	5.3
5	7.3	4.7	7.8	4.8	8.4	4.6	7.5	5.3
6	7.5	5.0	7.9	4.6	8.1	5.2	7.8	5.1
7	7.2	5.0	7.6	4.7	7.7	4.5	7.7	5.0
8	7.0	4.7	7.8	4.9	8.4	4.8	7.6	5.2
9	7.9	4.7	7.7	4.8	8.1	4.7	7.9	5.5
10	8.4	5.0	7.7	5.0	7.9	4.7	7.5	4.7
11	7.0	4.8	8.0	4.6	7.9	5.0	7.4	5.5
12	7.3	5.0	7.4	4.8	8.8	5.2	7.5	4.6
13	7.4	5.0	7.8	4.9	7.8	4.9	7.6	4.7
14	6.6	4.5	7.9	5.2	8.2	4.9	7.9	4.9
15	7.4	4.7	7.7	4.6	8.3	4.8	7.9	5.2
16	7.4	4.4	8.1	4.8	7.8	5.0	7.6	4.8
17	7.0	4.5	8.1	4.7	7.7	4.0	7.6	5.0
18	7.1	4.8	8.2	4.7	8.4	4.8	7.7	5.3
19	7.3	4.9	7.9	4.8	8.0	4.7	7.8	4.8
20	7.2	4.2	8.0	4.9	8.2	4.7	7.7	4.8
Total	146.5	93.9	157.0	96.0	161.6	95.7	153.7	101.6
Mean	7.325	4.695	7.85	4.80	8.08	4.785	7.685	5.08

H = seed height; *D* = seed diameter

From the analysis of the data in Table 1, it can be seen that the seed sizes are relatively large, generally ranging between 6.6 and 8.8 mm in height and 4.0 and 5.7 mm in diameter, respectively. From the analysis of the data recorded in the table, it results that the seeds of the red-flowered variety are, on average, the smallest in both assessed criteria ($H=7.325$ mm, $D=4.695$ mm). The seeds of the white-flowered biotype have the highest height ($H=8.08$ mm) and the seeds of the yellow-flowered variety have the highest average diameter ($D=5.08$ mm). The average sizes of the seeds practically correspond to the sizes of the plants of each variety, there being a certain correlation. The smallest, those with red flowers, develop plants with a height of 45-50 cm, while the white ones generate plants with a height of almost 90 cm. On average, the seed height/seed diameter ratio is 1.5975, very close to the golden number, the number of universal harmony, the divine proportion, which is 1.618. For the two characteristics studied, a summary statistic was also made based on the 160 measured values, from which the minimum, maximum, media, and standard deviation values resulted (Table 2).

Table 2. Summary statistics of height and diameter values of *M. jalapa* L. seeds

Statistical data	Height values	Diameter values
Mean	7.735	4.84
Standard Error	0.043686889	0.03382756
Median	7.8	4.8
Mode	7.8	4.7
Standard Deviation	0.390747418	0.302562892
Sample Variance	0.152683544	0.091544304
Kurtosis	0.410407235	0.845523527
Skewness	0.227172356	0.097982993
Range	2.2	1.7
Minimum	6.6	4
Maximum	8.8	5.7
Sum	618.8	387.2
Count	80	80

The seeds, separated by flower color, were kept in water for 24 hours for soaking and rehydration to improve germination, after which they were sown in plastic pots (40 alveoli for each variety in four replications) at the end of February.

Seed germination was observed, and germination (%) of *Myrabilis* varied significantly depending on the biotypes tested.

Seed germination was observed daily, and counting was performed after 6, 10, 15 and were terminated after 20 days of incubation period; final germination percentage was calculated and used for the interpretation of results (Table 3). The highest germination percentage of V1-biotype red flower was observed at 25°C.

Regarding the duration of germination, the determinations made showed that the first seedlings appeared after only 6 days after sowing, in all the varieties studied.

Table 3. The dynamics of *M. jalapa* plants' emergence

Variants (biotypes)	Seeds sown	Plants emergence	Plants emergence after*								Total lost plants no/%
			6days		10days		15days		20days		
			no	%	no	%	no	%	no	%	
V1- red flower	40	40 a	19	47.5	29	72.5	37	92.5	40	100.0 a	-
V2- pink flower	40	31 b	11	27.5	18	45.0	26	65.0	31	77.5b	9/22.5
V3- white flower	40	34 b	15	37.5	24	60.0	28	70.0	34	85.0 b	6/15.0
V4- yellow flower	40	38 a	16	40.0	27	67.5	32	80.0	38	95.0 a	2/5.0
Total	160	143	61	38.1	98	61.2	123	76.9	143	89.4	17/42.5
Mean	40	35.8	15.25	38.1	24.50	61.2	30.75	76.9	35.75	89.4	4.25/10.6

* Values marked by a different letter(s) are significantly different at $p < 0.05$, according to the LSD test

Analyzing Table 3, out of a total of 160 seeds sown in the 4 variants, it can be seen that 143 were viable, at a percentage of 89.4%, while 17 seeds did not germinate and were non-viable, at a percentage of 10.6%. Germination was staggered over a period of 20 days, with sowing taking place at the end of February.

The dynamics of emergence during the observation period showed important differences in terms of the number of emerged plants. After 6 days from the moment of sowing, variant V₁ (plants with red flowers) presents the most emerged plants, on average 19 plants, an aspect that is maintained in the other monitoring days. This is followed by V₄ (with yellow flowers), with the highest number of emerged plants, respectively 16. Variant V₂ (with pink flowers) recorded the lowest values, the number of emerged plants being only 11 plants.

After 20 days from sowing, all 40 seeds with red flowers germinated, while 17 seeds in the other three variants did not germinate, the lowest number of 2 non-viable seeds being in the variant with yellow flowers.

It is worth noting that after 10 days, except for V₂ with red flowers, more than half of the seeds were viable and most of the plants sprouted, most with red flowers (29 plants). The total germination rate peaked on the 15th day, respectively 92.5 % for V₁ (variant with red flower). The research showed that the germination rate of *M. jalapa* seeds decreased significantly with the prolongation of storage time, and the seeds germinated satisfactorily within 9 months. The germination rate peaked on day 6 (Li and Xu, 2022).

Germination (%) of *Mirabilis* varied significantly depending on the tested variants.

The highest germination percentage in the red color biotype was observed at variant V₁.

Analyzing the results obtained as a whole, it is found that after 10 days from sowing, an average germination of 61.2% is ensured, after 15 days of 76.9% and at the end of the period of 89.4%. In the variant with red flowers, all plants germinated, ensuring a percentage of 100%, followed by those with yellow flowers in a percentage of 95%.

With significant differences from these is the variant with pink flowers, in which the germination percentage reached only 77.5% and variant 3 with white flowers, with a percentage of 85%.

At the end of the germination period (after 20 days), not only the total germination was evaluated, but also the percentage of normal seedlings or seedlings with anomalies (deformed roots, cotyledons, or stems), respectively ungerminated seeds (Figure 3).

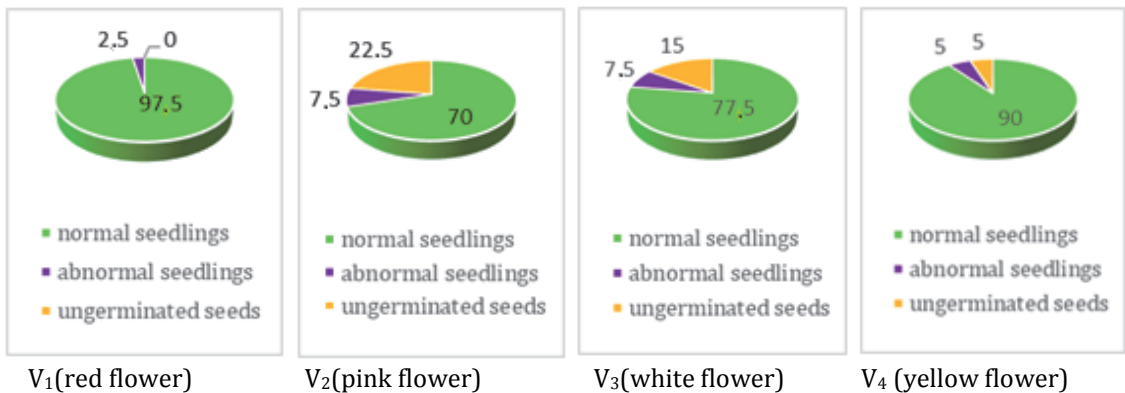


Figure 3. Seeds germination and seedling viability on *M. jalapa* (%)

The results show that out of the total number of germinated seeds, a certain percentage of the seedlings formed showed a series of anomalies that led to poor development and their premature death (Figure 3). These values ranged between 2.5 and 7.5%, respectively 1-3 seedlings from each variant.

The losses recorded after germination were 7.5% (3 seedlings each) for the variants with pink flowers (V₂) and white flowers (V₃), 5% (2 seedlings) for the variant with yellow flowers (V₄), and 2.5% (one seedling) for the variant with red flowers (V₁).

Results on plant growth and development

Observations and determinations were made on the obtained plants regarding their main characteristics: plant height and diameter, number of flowers per plant, flower diameter, and colour of flowers (Table 4; Figures 4 and 5).

Table 4. The main morphological characters of *M. jalapa* plants

Variants (biotypes)	Height/ plant (cm)	Diameter/plant (cm)	No. flower/ plant	Diameter flower (cm)
V1- red flower	55.4c	40.2c	123ab	3.5c
V2- pink flower	70.8b	50.4b	96c	3.5c
V3- white flower	110.3a	60.9a	110b	4.5a
V4- yellow flower	80.0b	60.8a	146a	4.0b
Mean	79.1	53.1	118.8	3.88

* Values marked by a different letter(s) are significantly different at $p < 0.05$, according to the LSD test

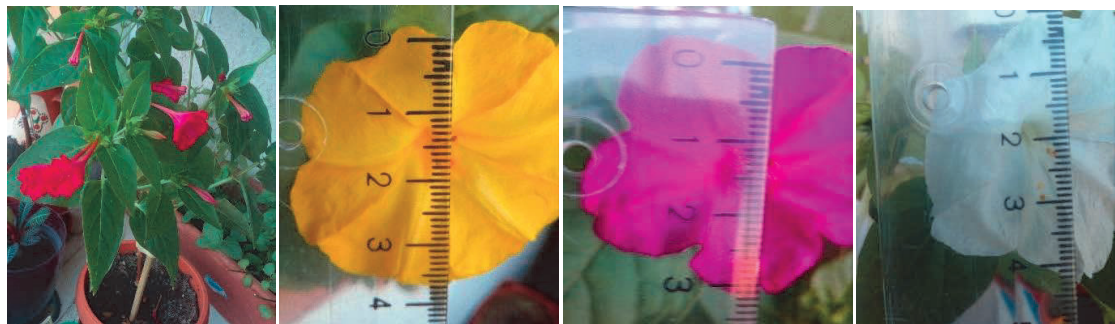


Figure 4. Diameter of flower (cm)

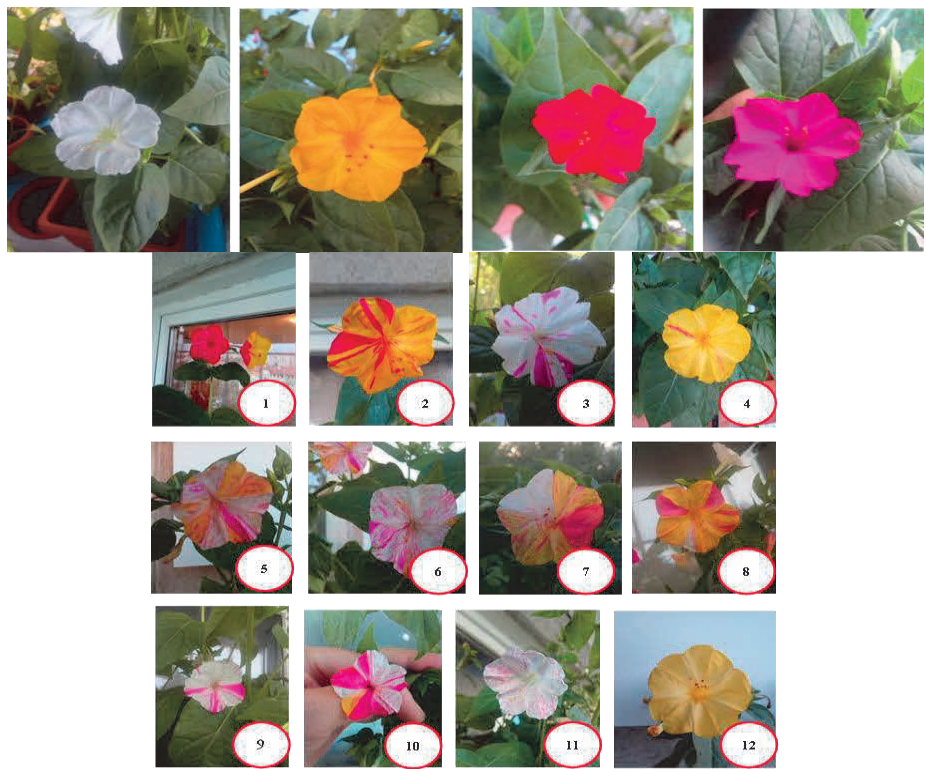


Figure 5. The color diversity of flowers of *M. jalapa* L.

Based on the data presented in Table 4, it was found that *M. jalapa* plants reached a height ranging from 55.4 cm for the red-flowered variant, which had the lowest height, to a maximum of 110.3 cm for the white-flowered variant, which was the most vigorous and also had the largest diameter, 60.9 cm. Regarding plant height, the statistical analysis of the data shows that there were significant differences between variants V_1 and V_3 and nonsignificant

differences between V_2 and V_4 . In the case of plant diameter, there were nonsignificant differences between variants V_3 and V_4 . The number of flowers per plant, during the maximum flowering period (August), had an average of 118.8 flowers. It is worth noting that the yellow-flowered variant had the highest number of flowers per plant (146 flowers), while the pink-flowered variant had only 96 flowers. The flowers at opening had a diameter ranging between 3.5 (red flower) and 4.5 (white flower). Regarding the number of flowers per plant, there are very significant differences between all the analyzed variants, and those regarding flower diameter are not significant between V_1 and V_2 .

The diversity of color combinations obtained is spectacular and amazing, from distinct colors on the same flower to color combinations and designs in the most varied forms (Figure 5).

CONCLUSIONS

This study highlights the potential of seed propagation of *M. jalapa* under greenhouse conditions, in plant pots, and some morphological aspects of the obtained plants. The results obtained highlight a good germination percentage of over 77%, reaching 100% for the red flower biotype, but there may also be small losses due to some anomalies. Monitoring the plants regarding their main characteristics (height, diameter, number of flowers) highlights some properties that justify their use for ornamental purposes.

Grown in pots, it can give terraces or balconies a touch of freshness and originality. Surprising colors and color combinations can be a good reason to cultivate it for those who are passionate about curiosities in the world of flowers.

Given the results of current studies on seed propagation, studies are needed, especially related to vegetative propagation through cuttings or tuber roots, focusing on future climatic conditions in the country.

M. jalapa has a strong environmental adaptability and reproductive capacity and deserves to be promoted and spread throughout the country.

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