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Flowering Biology of *Rubia tinctorum* L. under Mirzacho'l Conditions

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Abstract

The study of flowering biology is an important stage in the process of plant introduction, as it allows assessment of the degree of tolerance to environmental factors and provides recommendations for large-scale cultivation of introduced plant species. *R. tinctorum* is a perennial herb reaching 30 - 150 cm in height. The rhizome is elongated, creeping, branched, cylindrical, thick, nodular, and polycephalous. The stem is multiple, quadrangular, nodal, highly branched, and covered with rough hooked hairs. Medicinal preparations derived from its rhizome are used in the treatment of urinary calculi, kidney stones, gallstones, and gout, while in the textile industry the plant serves as a valuable dyeing raw material.

Keywords

Rubia tinctorum L., Medicinal Plant, Gout, Dye, Flowering Biology, Rhizome, Introduction, Reproduction

1. Introduction

In the Presidential Decree of the Republic of Uzbekistan dated April 10, 2020, No. PD-4670, "*On measures for the protection of wild-growing medicinal plants, their cultivation under controlled conditions, processing, and the rational use of available resources*", it was emphasized that the introduction and domestication of medicinal plants are among the most urgent issues [1]. Furthermore, the Development Strategy of New Uzbekistan (Goal 62) sets the task of "*increasing the share of domestically produced pharmaceuticals and medical products to* 80%" [2]. In achieving these objectives, the introduced species *Rubia tinctorum* L. (dyer's madder) occupies a special place in traditional medicine [3]-[5]. Numerous studies

abroad have been devoted to the investigation of its medicinal properties and the identification of bioactive compounds within the plant [6] [7].

Nowadays, as a result of the activities of industrial enterprises, the extraction and use of minerals, and other anthropogenic factors, chemical pollution of the soil cover, changes in soil properties, and productivity are observed all over the world. Various types of soil pollution lead to soil degradation, a decrease in the quality and quantity of productivity, as well as the formation of other problems related to the ecosystem [8].

The genus name *Rubia* was given by Pliny, derived from the Latin word *ruber* ("red"), due to the red dye obtained from the plant's roots. The species epithet *tinctorium* means "dyer", referring to its use in dyeing. During the 17th-18th centuries, when the use of red dye was widespread, the plant was extensively utilized. In Central Asia, *R. tinctorum* is widely distributed, and its roots serve as the source of the alizarin dye. Medicinal preparations derived from the rhizome are used in the treatment of urinary calculi, kidney stones, gallstones, and gout, while the roots and rhizomes are also employed as raw materials in the textile industry for dye production [9]-[11].

The study of flowering biology is a crucial stage in the introduction process, as it enables the assessment of plant tolerance to environmental factors and provides recommendations for large-scale cultivation of introduced species [12]. The ability of introduced plants to undergo reproductive processes—specifically flowering and seed formation—under new soil and climatic conditions is considered an important indicator of their adaptability [13].

In the unique agro-climatic conditions of the Mirzacho'l region, data on the flowering rhythm of Rubia species have not been previously studied. Therefore, in this research, we investigated the flowering dynamics of *Rubia tinctorum* L. cultivated under experimental field conditions in the Mirzacho'l environment.

2. Object and Methods of Research

The object of study was *R. tinctorum* (dyer's madder) introduced under the conditions of the Mirzacho'l steppe. This perennial herb reaches a height of 30 - 150 cm. Its rhizome is elongated, creeping, branched, cylindrical, thick, nodular, and polycephalous. The stems are multiple, quadrangular, nodal, highly branched, and covered with rough hooked hairs. The leaves are lanceolate to ovate, glossy, with thick veins on the lower side covered by hooked rough hairs, arranged in whorls of 4 - 6 along the stem with very short petioles. The fruit is a fleshy drupe, containing 1 - 2 seeds, initially red and later turning black. Flowering occurs from June to August, while fruit ripening takes place between August and September.

In studying the bioecological characteristics of the introduced species, we used the methodological guide "*Methods of Introduction Studies of Medicinal Plants*" published by I.V. Shilova *et al.* [14] and the methodological recommendations for conducting research from the discipline "*Plant Introduction*" developed by I.V. Belolipov *et al.* [15].

Field studies were carried out on the farms "Olmos Sehri" and "Qarapchi Kamoloti" of the Boyovut district, as well as in the territory of the Sirdaryo State Forestry. The selected region is characterized by sharply continental and arid climatic conditions. The annual average temperature is −14˚C. The mean temperature in January ranges from −6˚C in the north to −2˚C in the south. Winters are severe, with air temperatures dropping to −18˚C (down to −22˚C in Gulistan). According to the Sirdaryo Regional Hydrometeorological Center, precipitation in the Mirzacho'l region is low: 200 - 300 mm annually in the plains and 310 - 428 mm in the foothills, with the majority occurring in spring. The "Bekobod wind" in the plains reaches a velocity of 20 - 25 m/s, occasionally increasing to 40 m/s in Boyovut district, which enhances evaporation and accelerates soil salinization processes.

The flowering biology of the species was investigated using methodological guidelines published by O.A. Ashurmetov and Kh.K. Karshibayev [16]. The formation and growth of buds, as well as their opening mechanisms, were examined in 20 buds selected from the middle part of the plant shoots. The collected data were processed using Microsoft Excel software.

3. Results and Analysis

The flowers are small, yellowish-green, up to 1.5 cm in diameter, located in the axils of the leaves and at the tips of stems and branches, forming racemose or semi-umbellate inflorescences. They are bisexual, entomophilous, and in our region they most often consist of 4 floral organs, forming small racemose clusters. The calyx is indistinct; the corolla consists of five united petals, funnel- to rotate-shaped; the androecium comprises five stamens; the ovary is inferior, bilocular. Pedicel length is highly variable and largely depends on the number of buds formed in the leaf axil. The flowers are actinomorphic. The ovary is bilocular with a distinct style and bifid stigma.

The stamens are slightly shorter than the corolla. The studied plants belong to the group of species with flowers that open both during the day and at night. Each flower remains open for 7 - 9 days. The species is heliophytic.

Phenological observations were conducted from seed germination to fruit ripening. The beginning of a developmental phase was considered when 10% of plants exhibited that stage, while the full phase was recorded when 75% of plants had entered the stage [17].

Flowering and seed formation of introduced plants under new conditions serve as important indicators of adaptation. Only when the ecological requirements of plants coincide with the new environment do they flower, produce seeds, and ultimately leave progeny [18].

It is well known that bud development, from initiation to transformation into a flower, passes through several stages. O.A. Ashurmetov and B.A. Normatov (1998) distinguished nine stages in the bud development of sainfoin (*Onobrychis* spp.) [19].

The seasonal rhythm of development was studied by applying standard methods, observing the timing of the onset of the main developmental phases. In our research, we used the indicators proposed by O.A. Ashurmetov and B.A. Normatov (1998) [19].

Our observations on *R. tinctorum* demonstrated that under Mirzacho'l conditions, buds reach the pre-anthesis stage, *i.e.*, the stage of readiness for flowering, within 11 - 12 days. In particular, the bud formation stages (I-VII) in *R. tinctorum* last on average 9 - 10 days, and on the 11th day the bud enters stage VIII—the pre-anthesis phase.

At stage VIII, the average bud size was 13.6 ± 0.2 mm, while at stage IX, the flower size reached 14.8 ± 0.3 mm. Approximately half of the flower length consisted of the pedicel. The pedicel length ranged between 6 and 8 mm.

The Flowering Process. The process of flower opening is highly complex and can only be studied in natural conditions [20]. In agriculture, studying flowering is essential for developing strategies to increase plant productivity, optimize crop management, and regulate flowering time. In particular, determining the exact period of flowering enables proper crop care and enhances yield. Therefore, the study of "flowering biology" represents an integral component of reproductive biology and serves as a basis for genetic and breeding programs [21]-[23].

According to V.R. Chelak (1991), "*the process of flower opening and its duration define the biology of the species*" [24]. The reproductive system of each species is determined, on the one hand, by the morphological structures of the flower that ensure a specific type of pollination, and on the other hand, by functional aspects closely linked to floral biology [19]. It is well established that the floral biology of medicinal plants remains one of the least studied areas.

In *R. tinctorum*, bud opening begins with the expansion of the corolla lobes. During the course of our research, the flowering dynamics of the species were studied. The flowering process was observed in three phases: the beginning of flowering, full bloom, and the end of flowering. Light and air temperature played critical roles during anthesis: on sunny days, more flowers opened, whereas on cloudy and rainy days, flower opening was almost completely absent. Flower opening was accelerated as temperatures increased. The seasonal dynamics of flowering in this species were also studied. In desert environments, sudden rises or drops in air temperature had a notable effect on the flowering biology of the species. Similarly, during seasonal flowering, the increase in air temperature enhanced flower opening, whereas temperature declines reduced it.

At the stage of bud formation, *R. tinctorum* buds were initially very small (1.5 - 2.1 mm). After 7 - 10 days, the buds expanded to 2.8 mm, the upper parts of the petals began to separate, and petal lobes became visible between the calyx teeth. Our findings indicate that under Mirzacho'l conditions, *R. tinctorum* transitions to the generative phase of ontogenesis during the first year of vegetation. The flowers are arranged in 4 - 6 whorls along the inflorescence axis, with each whorl containing 10 - 16 flowers.

Research conducted between 2023 and 2025 demonstrated that the lifespan of a single flower lasted 7 - 9 days, an individual inflorescence bloomed for 17 - 24 days, and the flowering period of a single plant extended to 60 - 64 days. *R. tinctorum* is a heliophytic plant, and its flowers belong to the group of species that open during the daytime (Table 1).

Table 1. Phenology of flowering of *R. tinctorum* under Mirzacho'l conditions.

Observed years	Budding	Phases of flowering			Duration of flowering (days)
		Onset of flowering	Full flowering	End of flowering	
2023	04.06	13.06	21.06 - 24.07	03.08	60
2024	20.05	31.05	07.06 - 13.07	22.07	64
2025	08.05	19.05	26.05 - 02.07	09.07	63

In our scientific observations, the flower buds of individual *Rubia tinctorum* L. plants began to open predominantly during the third decade of May, while the peak flowering period occurred from the first decade of June to the third decade of July. The flowering duration lasted for 60 days in 2023 and extended to 63 days in 2025. The full (mass) flowering phase of *R. tinctorum* was observed approximately 9 - 12 days after the initial flower opening.

As noted above, in the first and third years of vegetation, the onset of the generative phase in plants was advanced by approximately 26 - 28 days. In the first year of vegetation, flowering began in the first decade of June, and at that time, the daily flowering dynamics were recorded as follows.

On June 13, at 08:00, when the air temperature was 26.2˚C and the relative humidity was 25%, 8 flowers had opened on a single plant. At 10:00, with an air temperature of 30.5˚C and a relative humidity of 20%, 16 flowers had opened. By 12:00, when the air temperature reached 33.5˚C and the relative humidity decreased to 19%, the number of open flowers per plant increased to 21. At 14:00, with an air temperature of 37.4˚C and a relative humidity of 17%, 19 flowers were recorded. At 16:00, when the air temperature rose to 38.1˚C and the relative humidity dropped to 15%, 8 flowers were observed. Finally, by 18:00, when the air temperature was 37.5˚C and the relative humidity had fallen to 14%, only 1 flower remained open per plant (Figure 1).

In the first year of vegetation, mass flowering occurred in the third decade of June, and at that time, the daily flowering dynamics were recorded as follows.

On June 21, at 08:00, when the air temperature was 27.6˚C and the relative humidity was 24%, 31 flowers had opened on a single plant. At 10:00, with an air temperature of 32.1˚C and a relative humidity of 19%, the number of open flowers increased to 65. By 12:00, when the air temperature reached 35.7˚C and the relative humidity decreased to 17%, 85 flowers were recorded per plant. At 14:00, with an air temperature of 37.4˚C and a relative humidity of 16%, the number of open flowers decreased to 45. At 16:00, when the air temperature rose to 38.3˚C and the

relative humidity dropped to 15%, 32 flowers were observed. Finally, by 18:00, when the air temperature was 38.2˚C and the relative humidity had fallen to 14%, 28 flowers were recorded on a single plant (Figure 2).

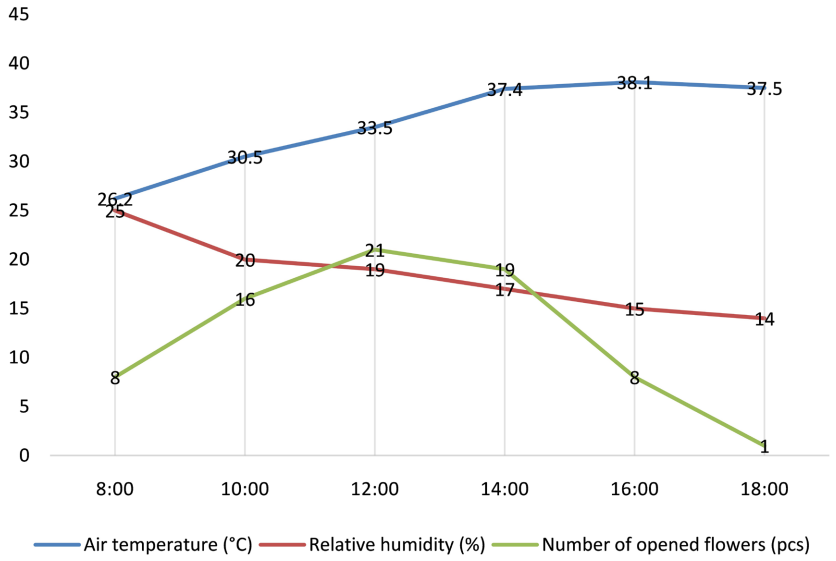

Figure 1. Diurnal flowering dynamics of *R. tinctorum* on the first day of flowering under Mirzacho'l conditions.

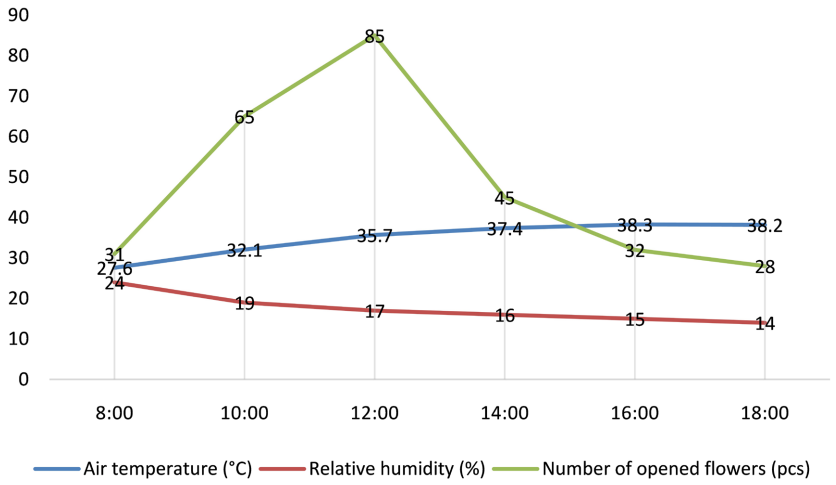

Figure 2. Diurnal flowering dynamics of *R. tinctorum* during the full blooming period under Mirzacho'l conditions.

In the first year of vegetation, the final phase of flowering occurred in the first decade of August, and during this period, the daily flowering dynamics were as follows.

On August 3, at 08:00, when the air temperature was 27.0˚C and the relative humidity was 32%, 2 flowers had opened on a single plant. At 10:00, with an air temperature of 32.2˚C and a relative humidity of 23%, 7 flowers were recorded.

By 12:00, when the air temperature reached 35.2˚C and the relative humidity decreased to 18%, 9 flowers were observed. At 14:00, with an air temperature of 37.6˚C and a relative humidity of 15%, 6 flowers had opened. At 16:00, when the air temperature was 38.0˚C and the relative humidity was 15%, no flowers opened. Similarly, at 18:00, with an air temperature of 36.0˚C and a relative humidity of 16%, no flower opening was observed (Figure 3).

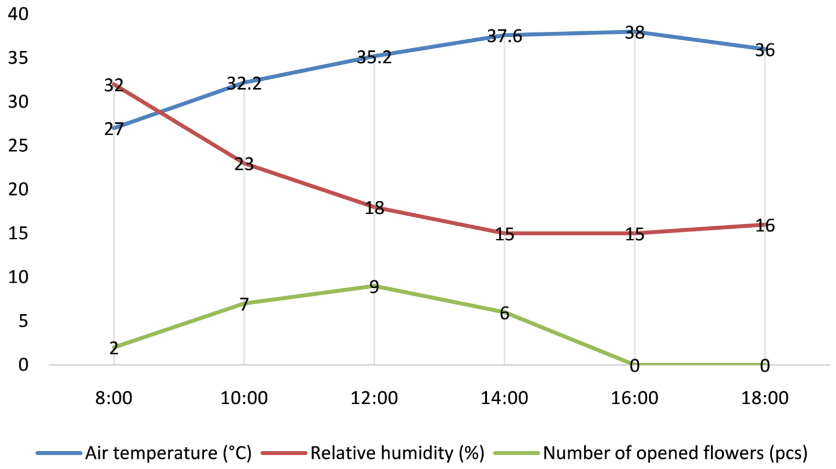

Figure 3. Diurnal flowering dynamics of *R. tinctorum* during the last days of the flowering phase under Mirzacho'l conditions.

The diurnal flowering dynamics of *R. tinctorum* were studied during the first year of the vegetation period (2023) (Table 2). For this purpose, the number of open flowers per plant was recorded. Our investigations revealed that the daily flowering period extended from 08:00 to 18:00. During the course of the day, the highest number of open flowers was observed between 12:00 and 14:00, with the flowering process reaching its peak at 12:00 (Table 2).

Table 2. Flowering biology of *R. tinctorum* under the climatic conditions of Mirzacho'l (2023).

Flowering period	Opened flowers	Observed hours						Per day
		8^{00}	10^{00}	12^{00}	14^{00}	16^{00}	18^{00}	
Beginning	pcs per plant	9	16	21	19	8	1	74
	%	12.16	21.62	28.37	25.67	10.81	1.35	100
Full flowering	pcs per plant	31	65	85	45	32	28	286
	%	10.83	22.72	29.72	15.73	11.18	9.79	100
End of flowering	pcs per plant	2	7	9	6	-	-	24
	%	8.33	29.16	37.5	25	-	-	100

R. tinctorum is an entomophilous plant, with the pollination process primarily carried out by insects. The dyer's madder is pollinated by honeybees, wild bees,

bumblebees, long-snouted beetles, butterflies, and other hymenopterans. The pollination process begins at 07:00 and continues until 18:00 (Figure 4).

Figure 4. Flowering phase of *R. tinctorum*.

The duration of the flowering period usually depends on the prevailing air temperature and atmospheric humidity of the region. The flowering period of plants is influenced by their origin, biological characteristics, age, and whether the spring season arrives early or late. The ability of plants to produce fruits and seeds under new conditions is one of the indicators of their successful adaptation, as it represents the fundamental outcome of the plant's life activity. Genetic adaptation to new environmental conditions becomes fixed in the subsequent generation of plants propagated by seeds, which in turn provides broad opportunities for selection. The transition of plants into the flowering and seed-setting phase is not determined by a single factor alone, but rather by the complex influence of ecological factors.

According to the data of Kh.Q. Qarshiboyev and colleagues, research in the field of plant introduction enriches the composition of flora, ensures the effective use of natural plant resources, and allows the selection and cultivation of plant species beneficial for the population and industry of our republic. Such research also expands breeding activities, facilitates the creation of promising new plant varieties, and ultimately contributes to ensuring food and ecological security in our homeland [23].

Thus, based on ecological indicators, *R. tinctorum* demonstrates relatively high tolerance to saline soils, highlighting its adaptability to saline conditions.

During our research, the flowering biology of *R. tinctorum* was studied in areas

of the Mirzacho'l region with both high soil salinity (EC 4 - 15 dS/m) and low salinity (EC below 2 dS/m, considered normal soils). As a result, in highly saline soils, flowering occurred from the first ten days of June until early August. Due to slower initial growth, flowering was observed to be slightly delayed (by approximately 1 - 2 weeks on average). In contrast, in soils with low salinity levels, flowering began in June, and seed maturation was observed in September.

4. Conclusions

The results of the conducted research revealed that the flowering process of *Rubia tinctorum* L. is directly influenced by its biological characteristics and the climatic factors of the Mirzacho'l region. It was determined that under the continental climatic conditions of Mirzacho'l, *R. tinctorum* L. is capable of entering the generative phase during the first year of vegetation, which indicates its high ecological adaptability. The flowering period was recorded to last an average of 60 - 64 days, suggesting that this species possesses a relatively long reproductive activity phase.

Phenological observations confirmed that *R. tinctorum* L. belongs to the group of diurnal flowering plants, with the peak of flower opening occurring predominantly on sunny days between 12:00 and 14:00. It was established that at the beginning of flowering, a single plant produced an average of 74 flowers, up to 286 flowers during full bloom, and about 24 flowers toward the end of the flowering period. These findings demonstrate that the intensity of flowering and the allocation of resources vary throughout the flowering phase.

During the pollination process, entomophily (insect-mediated pollination) was predominantly observed, indicating the effective ecological interaction of *R. tinctorum* L. with its pollinating agents.

Overall, the flowering biology indicators of *Rubia tinctorum* L.—including the duration of flowering, daily flowering rhythm, and entomophilous pollination traits—confirm the species' high adaptability to the environmental conditions of the Mirzacho'l region. The plant's ability to enter the generative phase within a short period, its intensive flowering capacity, and efficient pollination process serve as biological foundations for its stable growth and successful cultivation under local conditions.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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