



Phenological Stages of Development of *Tribulus terrestris* L. (*Zygophyllaceae* R. Br.) Under the Conditions of the Thracian Lowland Floristic Region of Bulgaria

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Abstract

The phenological stages of *Tribulus terrestris* L. in the Thracian Lowland floristic region of Bulgaria were studied. Growth characteristics of the species, the length of the vegetation period and the duration of each phenological stage were studied in three consecutive years. A detailed phenological spectrum was presented. A direct relation was established between the duration of the phenological stages, the calendar periods and the climatic features of the respective year.

Keywords: *Tribulus terrestris*, phenological stages, medicinal plant, steroidal saponins

Introductions

Tribulus terrestris is a medicinal plant of *Zygophyllaceae* R. Br. family, used in folk medicine in different countries by a number of generations. The major group of the biologically active substances determining the therapeutic properties of *Herba Tribulus terrestris* is that of steroidal saponins. Based on literature data and on the results of their own studies, Kostova et al. (2002) and Kostova and Dinchev (2005) reached to the conclusion that the saponins show different profiles in the different parts of the world where phytochemical studies have been carried out on *T. terrestris*. The above-cited authors mentioned that the steroidal

saponins in the herbal populations from Bulgaria are protodioscin (PD) and prototribestin (PT). Dinchev et al. (2010) pointed out that the standard Bulgarian products from *T. terrestris*, offered on the market, have the highest content of protodioscin, which was also confirmed by Semerdjieva (2014). The author found out in her study that the chemical content of the Bulgarian raw material produced from the plant, is of high

specificity and it has a high content of active furostanolic saponins.

Plant extracts improve the muscular tonus; they have diuretic, antiseptic and soothing effects and a general biostimulating effect. They also help treat

sterility in men, they increase sexuality, thus increasing the blood circulation and the supply of oxygen to human body (Arsyard, 1996; Adimoelja and Adaikan, 1997; Ganzera et al., 2001; Kostova and Dinchev, 2005; Pandey and Sainis, 2007; Moradi kor et al., 2013).

At present, a number of pharmaceutical products and food additives are produced from that medicinal plant all over the world and they are offered on the market (Dinchev et al., 2008). The following standard products based on *Tribulus terrestris* have been developed in our country: Tribestan of Sopharma, Tribocard of Bulgarian Rose Sevtopolis, ActaStyle[®] EroFort of Actavis, Vemoherb T (Vemo 99, Bulgaria), TribestaVit (Vigor AS CO, Bulgaria), EroFort (Actavis, Bulgaria), Tribistol, Andro-6, etc. The growing interest in and the market demand for products from *T. terrestris*

in our country and on the international market, determine the relevance of the research problem. The supply of adequate amounts of high quality raw material for the pharmaceutical industry in the country and for export is of great economic importance. As it is known, excessive exploitation and non-compliance with the rules for herb collection, place under threat of destruction and extinction the natural habitats of many medicinal plants (Hardalova, 1997; Hardalova et al., 1998; Evstatieva and Hardalova, 2004), including *Tribulus terrestris*. Biodiversity conservation and sustainable use of plant resources is of utmost importance for Bulgaria and for the whole world. That necessitates the cultivation of many medicinal plants, including the studied species, and its introduction either in polyculture or in culture.

For solving those problems related to the preservation of the resources of that species, it is extremely important to study in detail its biology and the duration of the phenological stages, in order to develop a scientifically based technology of growing it.

Materials and Methods

Tribulus terrestris var. *terrestris* was the object of study in the present investigation. According to the geographical division in "Flora of the Republic of Bulgaria", the Thracian Lowland floristic region is situated in the southern part of the transitional mountain-valley area and covers the large fields of Pazardzhik and Plovdiv in the west, Stara Zagora in the east and Haskovo in the south-east (Koprlev et al., 2002). In the present investigation, we subdivided the studied floristic region into the following sub-regions for a better orientation: Pazardzhik and Plovdiv field, Karlovo valley, Stara Zagora field and Haskovo field. In relation to the diverse climatic characteristics in the Thracian Lowland, ecological characteristics of the habitats of each population of the medicinal plant *T. terrestris* in the sub-regions were developed, including data about the sea level height, exposure, slope with the GPS system "ETREX LEGEND-HCX" (Table 1; 2; 3;4).

Temperatures and rainfalls in the sub-regions (Pazardzhik and Plovdiv field, Haskovo field, Karlovo valley and Stara Zagora field) in the Thracian Lowland are presented using data of the Institute of Meteorology and Hydrology at the Bulgarian Academy of Sciences, Plovdiv Branch. Diagrams of temperature and rainfall dynamics in mm/m² were made and compared with the norms for the respective regions taken from the Climatic Reference Book about temperature norms and rainfalls (Kyuchukova, 1983; Koleva and Peneva, 1990), (Fig. 1.).

Growth and development rhythm was determined by the methods of Beideman (1954) and Schultz (1966). Observations were carried out in the period June-October 2009-2012. In the months June-August the observations were conducted every third day and in the rest of the period – every 10th day.

Plant development is characterized by the following phenological stages: vegetation (**V**), buttoning (**B**), flowering (**F**), fruit-setting (**FS**) and plant dying (**PD**). According to the methods used, each of the stages was presented by the first letter of the respective stage and by a figure when divided into sub-stages, namely: **V, 1.** – Plant emergence. **V, 2.** – Formation of cotyledons; **V, 3.** – Formation of the initial stem and the first pair of true leaves; **V, 4.** – Branching and leaf formation; **F, 1.** – Beginning of flowering; **F, 2.** – End of flowering; **FS, 1.** – Beginning of fruit setting (coinciding with perianth abscission); **FS, 2.** – Fruit maturation (mass fruit setting – the first pair of leaves at the stem base turning yellow); **FS, 3.** – Full maturity (accompanied by slowed down growth and flowering); **PD, 1.** – Beginning of plant dying (the first pairs of leaves turn yellow and abscise and the stem color changes); **PD, 2.** – Mass plant dying.

Results

Tribulus terrestris is an annual grassy plant, propagated by seeds (terrophyte).

The first mass emergence of the studied populations and the beginning of vegetation (represented by V, 1. in Fig. 2) in the first year of the study (2009) was reported after heavy spring rains in the middle of June (12 June) at average day-and-night temperatures of 21-23°C and an air humidity reaching the norms of 65-68%.

In the second year of the study (2010), due to the varying spring temperatures, the mass emergence of the species was registered about 10 days later (25-30 June), (Fig. 2.). In the last two years (2011-2012), due to the extremely low air and soil humidity, the mass emergence was reported on 10-15 July (Fig. 2.).

The beginning of plant emergence (beginning of vegetation) is defined by the emergence of the two cotyledons above the soil surface. A well-developed main root with smaller lateral rootlets grows vertically down into the soil.

Cotyledons are oval elliptical, typically creased down the centre at the apical part, coriaceous in texture, initially green and photosynthesizing, with small conical pappi. They are 8-9 mm long and 3-4 mm wide in average. A month after their appearance, the cotyledons remain at the stem base, already withered.

The observations showed that 5-6 days after the emergence of cotyledons (stage V, 2.- according to the adopted representation of the methods applied) the first pair of true compound leaves and the initial stem are formed (presented as V, 3.), (Fig. 2). The first pair of compound leaves remains at the stem base, it turns yellow and abscises at the end of the species development.

Leaves are compound, evenly pinnate, with oppositely arranged leaflets. The compound leaves are oppositely or consecutively arranged along the stem with a flower at their base. Our observations confirmed the arrangement of the leaves along the stem, described in previous studies of Fukuda (1982). After the emergence of the first pair of compound leaves, two, three to four stems are formed (the species form up to 7-9 stems), (V, 4.). Stems are simpodially branched and the apical bud is vital for a certain period of time, then enters a period of dormancy or finishes with a flower. Our investigations showed that stem branches are formed acropetally, i.e. the first being formed at the base and later developing up the stem to the tip. They are also simpodially branched and they surpass in size the previous one. The way of branching, established in the present study, corresponded to the growth rate and branching described for that species by Nuhimovskiy et al. (1979). That way of branching does not lead to plant dying or finishing of its development. During the field observations it was established that the stem length of puncturevine varies from several centimeters to 2.40 m from the place of rooting.

Initially, the vegetative stage of development, related to the formation of stems and leaves, is prevailing in the growth and development process, representing 6.33% of the phenological development. When the species forms the first 5-7 cm long stem, the first flower buds emerge, setting the beginning of the buttoning stage (B).

3-4 weeks after plant emergence, the flowers are formed (presented as F, 1). Once the stage of flowering starts, it continues until the end of the vegetation period (until October or even sometimes November). The full blooming period of a flower continues for 3-4 days. The percentage ratio of the buttoning stage till the end of flowering of the first flower was calculated to be 4.22% of the whole phenological calendar period. Generally, the mass flowering in 2009 was reported from 27 June till 1 July and for the rest of the study years it was 10-15 days later. According to the methods used, perianth withering (presented as F, 2.) marks the end of the flowering stage and the beginning of the fruit setting stage (obviously observed fruitlets), (FS, 1). The

processes of fruit formation are related to changes in their size, colour and texture. Our observations confirmed that first the separate wedge-shaped nutlets are formed, meanwhile their colour and firmness are changed (fruit maturation). Fruit maturation goes on for at least 10-15 days (FS, 2.) after fruit setting. At the end of the species development, fruits enter the full maturity stage (FS, 3.). Full maturity of a single fruit is reached for 15-20 days after the initial process of maturation, however, under unfavourable conditions it is not always reached. Reaching full fruit maturity lasts for a month to a month and a half and it is directly related to temperature values and rainfalls. Our observations showed that at the initial stages of fruit setting fruits are dark-green, hairy and the prickles (spines) are soft (springy). Full maturation is accompanied by changes in the pericarp colour, in the fruit size, in the size and firmness of the prickles. Mature burrs are straw-yellow in colour, covered with hairs, tubercles and firm prickles diverging at an angle of 30°. Mature fruits split into separate nutlets immediately under the plant. The sharp spines help the zoochoric and anthropochoric spread of the seeds.

Fruit ripening is accompanied by withering of the first leaves at the petiole base and the next ones up the petiole (PD, 1.), stopping of the stem branching process, changing of the stem colour and a decrease of the number new flowers.

Growth and development rhythm reflecting the phenological spectrum of *T. terrestris* is presented in Fig. 2.

Discussion

Growth and development of the species under the conditions of Bulgaria is characterized by a vegetation period from the end of May until October and, under favourable climatic conditions, it could continue even in November.

Growth and development of the populations of that species is quite susceptible to temperature values and soil and air humidity in the separate years. The climatic characteristic of the first years of the study showed that the average day and-night temperatures surpassed the norm by 1-2°C in the months of the active vegetation period of the species (June – August), i.e. 22.4°C (June), 24.6°C (July), 23.9°C (August) and the amount of rainfall was close to the seasonal norm – within 47-59.1 mm (the norm being 58-63 mm). The continuous high temperature sum values in a combination with the optimal humidity, created excellent conditions for the mass emergence and development of *T. terrestris*. In the second year (2010) data showed variable average daily temperatures for June (21.4°C), July (24.2°C) and

the most intensive rainfalls – Σ -424.2 mm (Pazardzhik), Σ -334.7 mm (Plovdiv), the highest amount of rainfall per m² being reported in June (120 versus the norm of 49).

Those climatic characteristics had a markedly unfavourable effect on the development of the studied species, especially at the initial phenological stages (germination and emergence). In the last two years of the investigation (2011-2012) high average day-and-night temperatures were reported: 27°C (July), 25°C (August) and 26.5°C (July), 25°C (August) and insignificant amount of rainfall. The total precipitation sum for the whole period of vegetation in 2011 was Σ 196.7-198.3 mm and in 2012 – Σ -78 mm. It should be noted that in July and August 2 mm of rainfall was recorded, compared to the norm of 31-35mm/m². Dry spells of weather, especially at the beginning of vegetation, had an important effect on the uniform emergence and growth of the species. The low water retention capacity of soil hampered the optimal emergence and development, which was confirmed by the lower number of plants per m² and their smaller size, established in some of the populations.

The study carried out confirmed that the effect of the climatic factors in the separate habitats was of a great importance for the mass germination and emergence of the species. Optimal conditions require continuous air temperatures of over 21°C combined with abundant rainfall. The observations showed that usually the first seed to emerge is the one close to the basal part of the nutlet and the rest seeds remain dormant. In case the environmental conditions continue to be favourable, the other seeds also emerge. That special adaptation is a means of prevention from bad climatic conditions and supports the survival of the species. Those conclusions from the observations are similar to the results of the studies carried out by Ernst & Tolsma (1988) and Nikolova (2010). Under unfavourable soil and climatic conditions – above all low temperatures or insufficient rainfall – germination is delayed and the period of emergence is prolonged until September-October.

It should be noted that following out the ontogenetic development of the species, it was established that after the beginning of vegetation, the phenological stages were going on at the same time. Stem growth and branching, formation of new leaves, flowering and fruit setting were running in parallel. That defines the species as a plant of unlimited growth and development. That statement is also confirmed by the fact that the different stages of the vegetative and generative development of the individuals are observed

throughout the whole phenological period. The vegetative and generative stages continue until plant death, i.e. until the first frosts, which come in that floristic region at the end of October but in separate years it may happen in September (Kyuchukova, 1983), (Koleva and Peneva, 1990).

Calendar periods and the duration of the stages are closely dependent on the natural characteristics of the sub-region. In the separate sub-regions of that floristic region, the differences are within 8-10 days. It is completely understandable by the different geographical locations. The sub-region of Karlovo valley is characterized by a longer spring season and lower temperature values compared to the other sub-regions. Taking into account those characteristics, the week delay in mass emergence and development of *Tribulus terrestris* L. compared to the other sub-regions, is easy to explain. The sub-region of Haskovo field is characterized by warmer springs with higher average daily temperatures, which favours the earlier emergence and vegetation of the species (a week before the other regions).

Although *Tribulus terrestris* is a typical thermophyte, the dry spell of weather and maximal air temperatures exceeding 42°C in July-August, as the case in the last two years of the study (2011-2012), had an unfavourable effect on its growth and development and it was a disaster for the local populations in some sub-regions. The result is the same when frosts come and permanent low temperatures below 5°C continue for more than 4-5 days. All the leaves and stems of the species turn yellow and the plants die. That marks the end of vegetation (PD, 2.). The average duration of a vegetation period for the floristic region is about 100-140 days – from June through October, and, in some warmer parts of the region – until November. The length of the vegetation period is determined by the climatic characteristics of the year.

In world literature, there are sparse data about the length of some vegetation stages of the species, mainly about flowering and fruit setting (Boydston, 1990, Ernst and Tolsma, 1988). No data about previous studies on the growth and development of the species in Bulgaria were found in the available scientific literature.

Conclusion

➤ Under the conditions of the Thracian Lowland floristic region of Bulgaria, growth and development of the species is characterized by the following vegetation period: from the end of May till October and under good climatic conditions it continues until November.

➤ The average length of the vegetation period is 100-140 days (from June through October) and depends on extreme high temperatures, as well as on temperatures dropping down to 5°C (first frost bites).

➤ The phenological development of the species shows the parallel running of the vegetative and generative stages, going on until the end of vegetation (October-November).

➤ Fruits are set 3-4 days after flowering and they mature for 30-45 days.

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Table 1. Distribution of *Tribulus terrestris* in sub-regions Karlovo valley.

Lokality	GPS Coordinats N/E	Sea level height	Slope in °	Soil type
village Marino pole-LH 21	N 42.58590 E 24.86690	339	6	Leached-maroon (Chromic luvisols / FAO)
vilage Vedrare-LH 21	N 42.55738 E 24.87640	339	26,23	Alluvial-meadow (Fluvisols/FAO)
City Banya-LH 21	N 42.54170 E 24.82420	295	4	Alluvial-meadow (Fluvisols/FAO)

Table 2. Distribution of *Tribulus terrestris* in sub-regions Stara Zagora field

Lokality	GPS Coordinats N/E	Sea level height	Slope in °	Soil type
Stara Zagora-LG 89	N 42.40850 E 25.61460	213	5	Humus-carbonizing (Renzinas/FAO)
Chirpan-LH 21	N 42.20708 E 25.34337	169	5	Humus-carbonizing (Renzinas/FAO)

Table 3. Distribution of *Tribulus terrestris* in sub-regions Haskovo field

Lokality	GPS Coordinats N/E	Sea level height	Slope in °	Soil type
City Simeonovgrad-MG 05	N 42.13630 E 25.58690	199	5	Smolnik and Alluvial – maroon (Pellic vertisols, Fluvisols /FAO)
Village Podkrepa -LG 04	N 42.03240 E 25.84080	144	7	Leached-maroon(Chromic luvisols /FAO)
South of the city Harmanli- MG 04	N 41.92670 E 25.68760	99	6	Leached-maroon(Chromic luvisols /FAO)
Village Bulgarian-MG 09	N 41.92587 E 25.92319	287	5	Leached-maroon(Chromic luvisols /FAO)
Village G.Dobrevo-MG 23	N 41.98291 E 26.01205	199	5	Alluvial-meadow (Fluvisols /FAO)
Village Raikova mogila-MG 42	N 41.84140 E 26.11570	199	5	Alluvial-meadow (Fluvisols /FAO)
City Svilengrad-MG 32	N 41.76991 E 26.13012	199	5	Alluvial-meadow (Fluvisols /FAO)

Table 4. Distribution of *Tribulus terrestris* in sub-regions Plovdiv and Pazardzhik field

Lokality	GPS Coordinats N/E	Sea level height	Slope in °	Soil type
Village Zlokuchene - KG67	E 24,17030 N42,21980	220	5	Fluvisols /FAO alluvium.
Village Ovchepoltsi-KG89	N 42,33300 E 24,40160	469	24,34	Leached-maroon, (Chromic luvisols/FAO)
Village Chernogorovo- KG36	N 42,27730 E 24,38850	332	20,88	Fluvisols and Chromic luvisols/FAO
Village Varvara-KG67	N 42,15890 E 24,14423	298	28,3	Alluvial-meadow (Fluvisols/FAO)
Glavinitsa-KG76	N 42,16240 E 24,32160	220	5	Alluvial-meadow (Fluvisols / FAO)
Pazardjik -KG77	N 42,21083 E 24,32863	205	6	Alluvial-meadow (Fluvisols / FAO)
Between city Rakovski and village Strqma-LH 11	N 42.2606 E 2490980	181	5	Alluvial-meadow (Fluvisols/FAO)
City Stamboliyski -KG77	N 42,15420 E 24,55110	199	##### #	Alluvial-meadow (Fluvisols/FAO)
City Sadovo-LG26	N 42,13750 E 24,94080	153	7	Meadow-marsh (Gleysols/Fao)
City Parvomay -KG56	N 42,11360 E 25,22260	155	9,04	Leached maroon (Chromic Luvisols/ Fao)

Fig. 1. Dynamics of average daily average temperatures and rainfalls in the Thracian Lowland Floristic Region of Bulgaria.

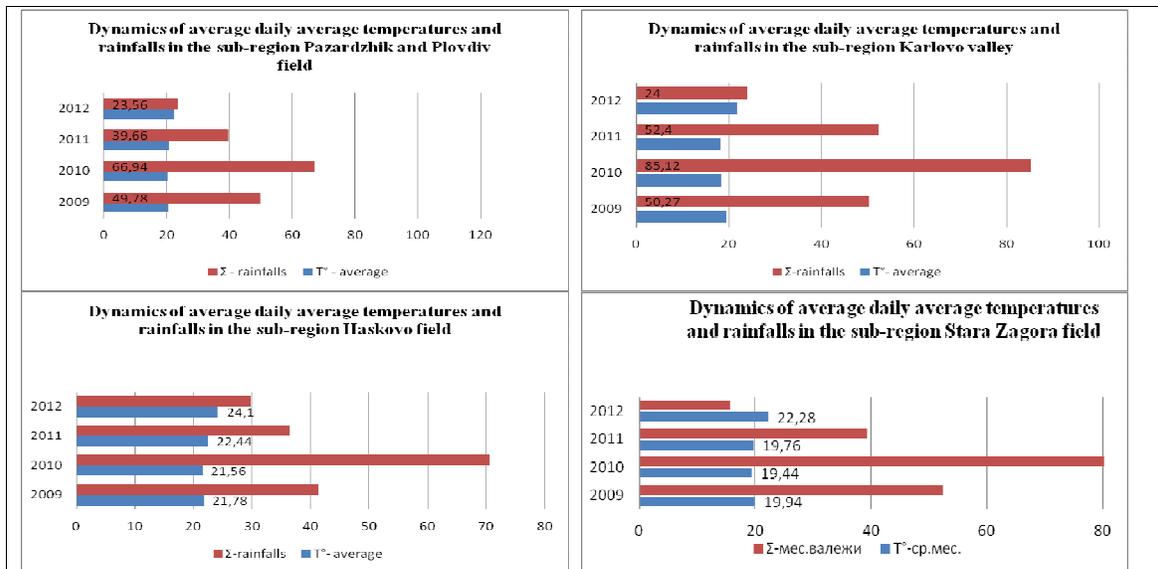
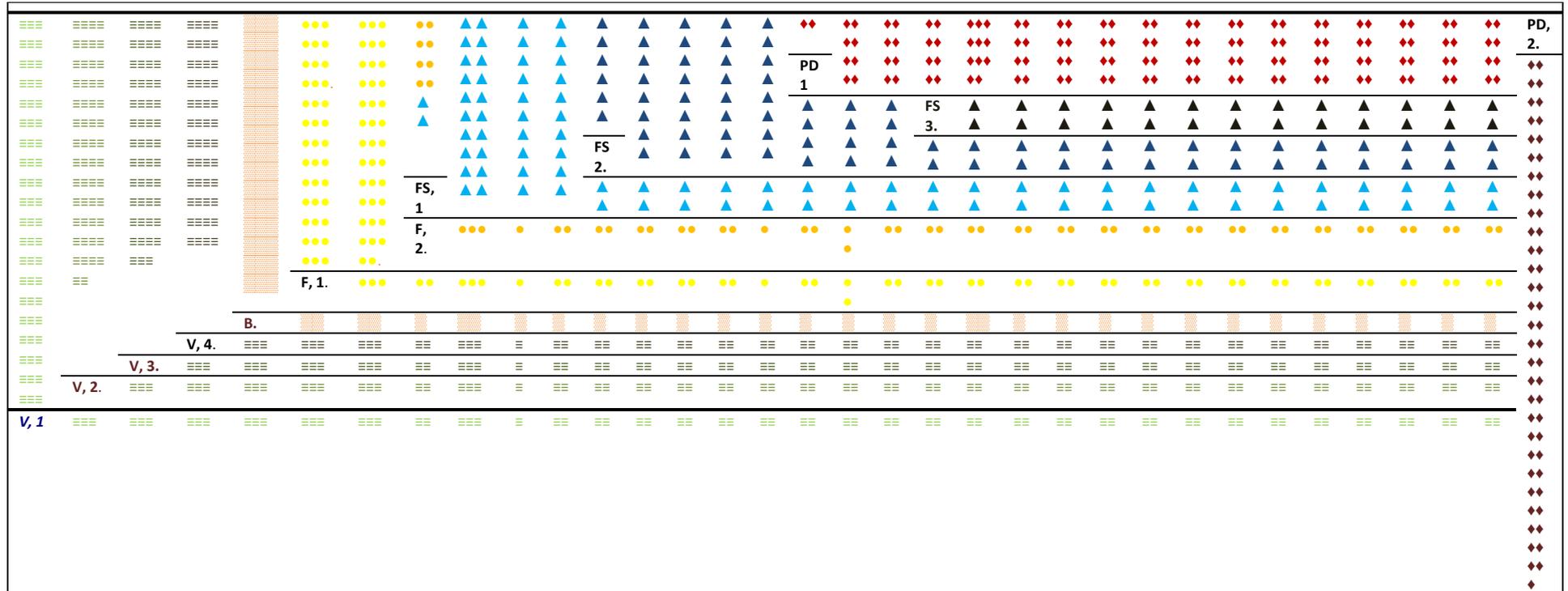


Fig. 2. Phenological stages of *Tribulus terrestris*



V-Vegetation
 V, 1. – Plant emergence
 V, 2. – Formation of cotyledons
 V, 3. – Formation of the initial stem and the first pair of true leaves.
 V, 4. – Branching and leaf formation.
B - Buttoning
 F, 1. – Beginning of flowering
 F, 2. – End of flowering
 FS, 1. – Beginning of fruit setting
 FS, 2. – Fruit maturation
 FS, 3. – Full maturity.
 PD, 1. – Beginning of plant dying.PD, 2. – Mass plant dying

V, 1. =====	B. =====	F, 1. ●●●	FS, 1. ▲▲▲	PD, 1. ◆◆◆
V, 2. =====		F, 2. ●●●	FS, 2. ▲▲▲	PD, 2. ◆◆◆
V, 3. =====			FS, 3. ▲▲▲	
V, 4. =====				
Vegetation	Buttoning	Flowering	Fruit-setting	Plant dying