Original Article

STRUCTURAL FEATURES AND GROWTH DEVELOPMENT OF HYSSOPUS OFFICINALIS L. IN TASHKENT AND JIZZAKH CONDITIONS

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Abstract

This article presents the results of scientific research on the growth and development of Hyssop officinalis species from the Lamiaceae family, the morpho-anatomical structure of the vegetative organs, the localization of biologically active substances, that is, the pharmacological activity, as well as the study of the chemical composition, under different conditions of introduction. Based on the study of the anatomical structure of the assimilative and shoot organs of the Hyssopus officinalis species in Tashkent and Jizzakh conditions, diagnostic signs specific to the species were determined. Also, on the basis of the comparative analysis of the anatomical signs in the vegetative organs, the structural and adaptive signs characteristic of the conditions of Tashkent and Jizzakh were determined.

Keywords: morphology, phenology, anatomy, vegetative organs, Hyssopus officinalis, introduction, Tashkent, Jizzakh.

INTRODUCTION

Today, the demand for extracting organic (medicinal) substances from the cells and tissues of medicinal plants by the method of biosynthesis is increasing day by day. One of the promising areas of development of pharmaceutical biotechnology is the creation of sources of medicinal plants with pharmacological effects.

There are about 600 medicinal plants and more than 500 essential oil-containing plants in the flora of Uzbekistan, of which more than 200 types of plants are included in the state pharmacopoeia for use in official medicine [13]. It is possible to develop the pharmaceutical industry through the cultivation of wild-spread local and introduced plants in order to search for and obtain new sources of biologically active substances contained in medicinal plants with the help of modern high technologies. Flavonoids, vitamins, essential oils contained in medicinal plants are considered valuable biological compounds and are widely used in the chemical, cosmetic, medical and food industries [1, 6, 7, 8].

The composition of plants belonging to the Lamiaceae family is especially rich in essential oils, and one of the goals of our scientific research is to ensure the wide use of essential oils in the pharmaceutical industry and to study the bioecological properties of introduced and cultivated plants such as marjoram, oregano, hyssop, lavender, and salvia. Essential oils have a pungent smell and are accumulated in the external (different types of glandular hairs, glandular spots, special glands) or internal (cavities, essential oil paths, channels) cells of plants [11]. The presence or absence of these substances depends on environmental conditions and is important in increasing the amount of raw materials. In the microscopy of plant organs, the cells that collect essential oils or other types of biologically active substances, their quantity is determined on the basis of scientific research.

Today, the plant is characterized by a number of useful properties and is widely used in folk medicine in many countries as an anti-inflammatory, wound healing and soothing agent, as an invigorator. Hyssop officinalis is famous for treating lung diseases, and herbal decoctions have been used for bronchitis, shortness of breath, tuberculosis, profuse sweating, and chest pain. The antimicrobial effect of the essential oils contained in the plant has been confirmed by scientific studies, and it helps well when rinsing in eye, mouth and throat diseases [10, 13].

One of the promising taxa is the growth and development of Hyssop officinalis of the Lamiaceae family under the conditions of introduction, their morpho-anatomical structure, adaptation features and the localization of biologically active substances, that istheir pharmacological activity, as well as their chemical composition have not been sufficiently studied, and very little information is provided in scientific literature sources.

The growth and development of Hyssop officinalis species in the conditions of introduction (Tashkent and Jizzakh), the morpho-anatomical structure of vegetative organs have not been studied, which shows the relevance and scientific novelty of our research.

The purpose of the study is to study the diagnostic and adaptive features of the Hyssopus officinalis species based on the growth and development of the Hyssopus officinalis species in the conditions of Tashkent and Jizzakh, as well as the anatomical structure of the assimilative and shoot organs, as well as to determine the localization of biologically active substances.

Materials and methods.

The object of research is Hyssopus officinalis introduced in Tashkent and Jizzakh region, which is a perennial bush. The stem is erect, four-sided, the base of the stem is woody, the tip is covered with unicellular simple trichomes, and the linear leaves are covered with glandular trichomes.

Seasonal development pattern of Hyssopus officinalis grown under introduction conditions in Tashkent Botanical Garden and Jizzakh region was reported and were made observations according to the method of Phenological by I.N. Beidemann [4].

Vegetative and generative periods were noted. Growth initiation, foal growth, leaf emergence, size and shedding during the vegetative period; the growth of branches, budding, flowering, formation of fruits, and their ripening were observed.

In 2020-2022, scientific research was carried out on the Hyssopus officinalis plant introduced to the climatic conditions of Tashkent Botanical Garden and Jizzakh region. Hyssopus officinalis was fixed in 70% ethanol in the flowering phase of the generative period in order to study the anatomical structure of vegetative organs (leaf and stem). The processes of preparing cuttings from vegetative organs were carried out by hand. Paradermal and transverse sections were prepared to study the anatomical structure of the epidermis and stomata in the leaf. In studying the structural features of the vegetative organs of the plant, transverse sections were prepared from the middle part of the leaf and the base of the stem. Sections were stained with methylene blue and safranin and sealed with glycerin-gelatin [3]. The main tissues and cells of assimilative and axis organs in plants K. Esau [16], N.S. Kiseleva [12], epidermis – S.F. Zakharevich [9], types of leaf stoma of M.A. Baranova it was described according to the methods [2]. Photomicrographs were made using a computer photomicroscope, a Canon A123 digital camera, and a Motic B1-220A-3 microscope.

Results and discussion.

The seeds of Hyssopus officinalis species were sown in the field in autumn, that isin the middle of October (13.X.2020) in 3 replicates, and seed germination was observed on the 15-17 days of the study. Seed germination under field conditions was 56%, the total germination of seeds was observed on 18-20 days of planting, and it was determined that the energy of seed germination was 52.1%.

When plant seeds were planted in laboratory conditions, the fertility of seeds stored for one year at temperature +20-22°C was 60.4±2.9%, and the fertility of seeds stored for two years was 53.1±2.5%. Fertilization of seeds stored for one year at room temperature +22-25°C was 69.5±2.0%, and the germination of seeds stored for two years at the same temperature decreased to 55.1±2.5% (Table 1).

Table 1 Hyssopus officinalis seeds stored for different periods fertility (%)

Temperature, ^{0}C	**Shelf life, months**			
	6	12	18	24.
20-22	43,5±2,6	60,4±2,9	58,5±3,1	53,1±2,5
22-25	52,8±2,8	69,8±2,0	62,5±2,9	55,1±2,5

Hyssopus officinalis species germinated in field conditions of the Tashkent Botanical Garden at the end of October (29.X.2019) and the seed leaves are round in shape, 0.2-0.3 cm long and 0.2-0.3 cm wide. The length of the seed leaf band was 0.3-0.4 cm, the hypocotyl was light green, 1.0-1.5 cm long, and the root was 1.5-1.7 cm.

On the 26-28th day of the study (14.11.2019), 2 small leaves with short bands were formed in the lawns. At the end of November, the growth of seedlings slowed down, and in this case, it finished its vegetation, i.e., wintered.

The growth of seedlings was observed from the third decade of March of the following year (2020). In the middle of April, the length of the shoots reached 1.5-2.5 cm, forming up to 3-4 leaves, the length of which was 0.5-1.2 cm and the width was 0.2-0.3 cm, the plant during the initial vegetation period, it grew and developed in a monopodial type. At this time, it is possible to observe the morphological characteristics of the leaves, i.e., the leaves are elongated-lanceolate in shape, covered with hairs on the outside, the edges of the leaf are slightly curved, and the main vein of the leaf protrudes from the bottom, that is the abaxial side of the leaf.

In the middle of May, the air temperature was 25-28 ºC, the height of the seedlings was 5-6 cm, and 7-8 new small leaves were formed on the stem. The stem has become slightly larger, and the lower part has begun to turn light brown. The roots are branched up to the II order, and the length of the main root reaches 3.5-4 cm. Seedlings were watered 2 times a week and humidity was maintained at the same rate.

At the end of June, the height of the seedlings reached 10-18 cm and produced 14-16 pairs of leaves. The differences between the large and small leaves of the plant are large, and their length is 1.5-4.5 cm, and the width is 0.3-1 cm. The edges of the leaf plate are mostly straight, or can vary with a slightly large dentate groove. The lower part of the stem became dark brown and enlarged, and the lower leaves turned yellow and fell off, replaced by lateral branches. The main roots deepened into the soil up to 10-12 cm and formed lateral roots of the III order.

In the middle of July, the length of side branches reached 4-8 cm, and it was found that there were 3-4 pairs of leaves. At the end of the month, the height of the sprouts reached 22-26 cm, and the plant was characterized by sympodial growth, 8-12 lateral (vegetative) branches were produced per bush. It was found that they form 8-10 pairs of leaves, and their size is smaller than the leaves formed on the main stem. The length of the main roots reached 13-15 cm, and the length of the secondary roots of the II and III order reached 7-10 cm.

In the middle of August, the main stems of the plants entered the generative period, budding, and the flowering phase began at the end of the month. The height of the plant reached 30-35 cm, the length of vegetative branches reached 12-15 cm. At this time, the root system of the plant developed rapidly and produced many lateral roots of the II and III-IV order.

In the third decade of September, plant seedlings were transplanted to a permanent place prepared in advance: the section of medicinal plants of the Tashkent Botanical Garden and Jizzakh region.

The development, flowering and fruiting of the transplanted seedlings slowed down a bit, and the generative period stopped.

Transplanted seedlings under both conditions revived somewhat in late October and remained above ground green until late November before frost, and annual shoots were completely dry in December.

In the first growing year of Hyssopus officinalis, the duration of vegetation was 180-200 days, the total generative period was 50-55 days. At the end of September 2020, the second year vegetation of Hyssopus officinalis seedlings (year 2021) transferred to the Tashkent Botanical Garden and Jizzakh region began at the end of March. The climatic conditions of the Jizzakh region are slightly different from the climatic conditions of the city of Tashkent due to the heat of the air and low humidity (air dryness). At the same time, their soil composition is also different, and the soil of the Tashkent Botanical Garden is considered a typical gray soil. The soil of Jizzakh region is light gray soil, weak and moderately saline [15].

In the second vegetation year, up to 8-10 generative branches formed in the first vegetation year grew and developed, and in the beginning of June, in the Jizzakh region, in the middle of this month, the plants grown in the Tashkent Botanical Garden entered the generative period. It was determined that the flowering phase of plants grown in the conditions of Jizzakh in the middle of the month of the generative period (10-12 days), and in the conditions of Tashkent at the end of June. The flowers of the plant are on the tip of the generative stem, forming spike-like inflorescences, which are located on one side of the stem. Spike inflorescences were 20-25 cm long, and each inflorescence produced 60 to 115 flowers (Fig. 1).

In the second growing year, the above-ground green mass of the plants increased, until the end of the growing season, an average of 35-40 generative branches were formed per bush. At the end of July, the ripening of the seeds on the first flowering generative stems was observed. The budding, flowering and fruiting phase continued until September on the newly formed generative branches on the plant.

In the second vegetation year, it was found that the duration of vegetation in both conditions is 190-200 days, and the total generative period is 85-95 days. During the vegetation of Hyssopus officinalis grown in the conditions of Tashkent and Jizzakh, the beginning and duration of the phases differed by 7-10 days, it was found that the growth and development of the plants, and the productivity of the above-ground part were almost the same.

a b

Figure 1. Seedlings of Hyssopus officinalis L. grown in a) Tashkent Botanical Garden and b) Jizzakh region (2021)

Based on the results obtained above, taking into account the differences in soil composition and climatic conditions of the Hyssopus officinalis species in the conditions of Tashkent Botanical Garden and Jizzakh, the anatomical structure of the assimilative and shoot organs of the Hyssopus officinalis plant in the conditions of Tashkent Botanical Garden and Jizzakh, structural diagnostic and adaptive signs of this species signs adapted to the conditions, as well as the localization of biologically active substances in the assimilative organs were determined.

A leaf is a vegetative organ of a plant and performs the functions of photosynthesis, transpiration and gas exchange.

The leaves of Hyssopus officinalis have a bilateral type in morphological structure, opposite, lanceolate, with a short leaf strip, the edges of the leaves are slightly bent from the underside. In the paradermal parts of the leaf, the cell walls of the adaxial epidermis are relatively wavy, while the cell walls of the abaxial epidermis are strongly wavy and polygonal. Adaxial epidermal cells have been found to be much larger than abaxial epidermal cells. In the epidermal cells of the leaf, simple unicellular and round multicellular essential oil plants are covered with glandular trichomes. Under the conditions of the Tashkent Botanical Garden, it was found that the adaxial and abaxial epidermal cells of the leaf are larger, and the rarity of simple unicellular and glandular trichomes prevails in the characteristics of mesophytic conditions. Under the conditions of Jizzakh, it was found that the abundance of adaxial and abaxial cells of the epidermis, consisting of small cells, the abundance of simple and glandular trichomes are the predominant xeromorphic features of these species adapted to xerophytic, that is, arid conditions (Figures 2, 3).

In both conditions, the leaves of the species Hyssopus officinalis have an amphistomatic structure. Petioles are located on both sides of the leaf blade and are located across the longitudinal axis of the leaf. Leaf stomata are two-way, round-oval in shape. Leaf stomata in the adaxial epidermal cells of the leaf are few and numerous in the abaxial epidermis. In the conditions of the Tashkent Botanical Garden, a small number of leafworms was found, and in the Jizzakh region - an abundance of leafworms. This is the abundance of leaf stomata in the conditions of the Jizzakh region, which, in turn, ensures less water leakage from the leaf epidermis (Figures 2, 3). The anatomical structure of the leaf of Hyssopus officinalis in transverse sections has a dorsiventral type [5], the columnar cells in the leaf mesophyll are located under the adaxial epidermal cells of the leaf, and the porous cells are located in the abaxial part of the leaf.

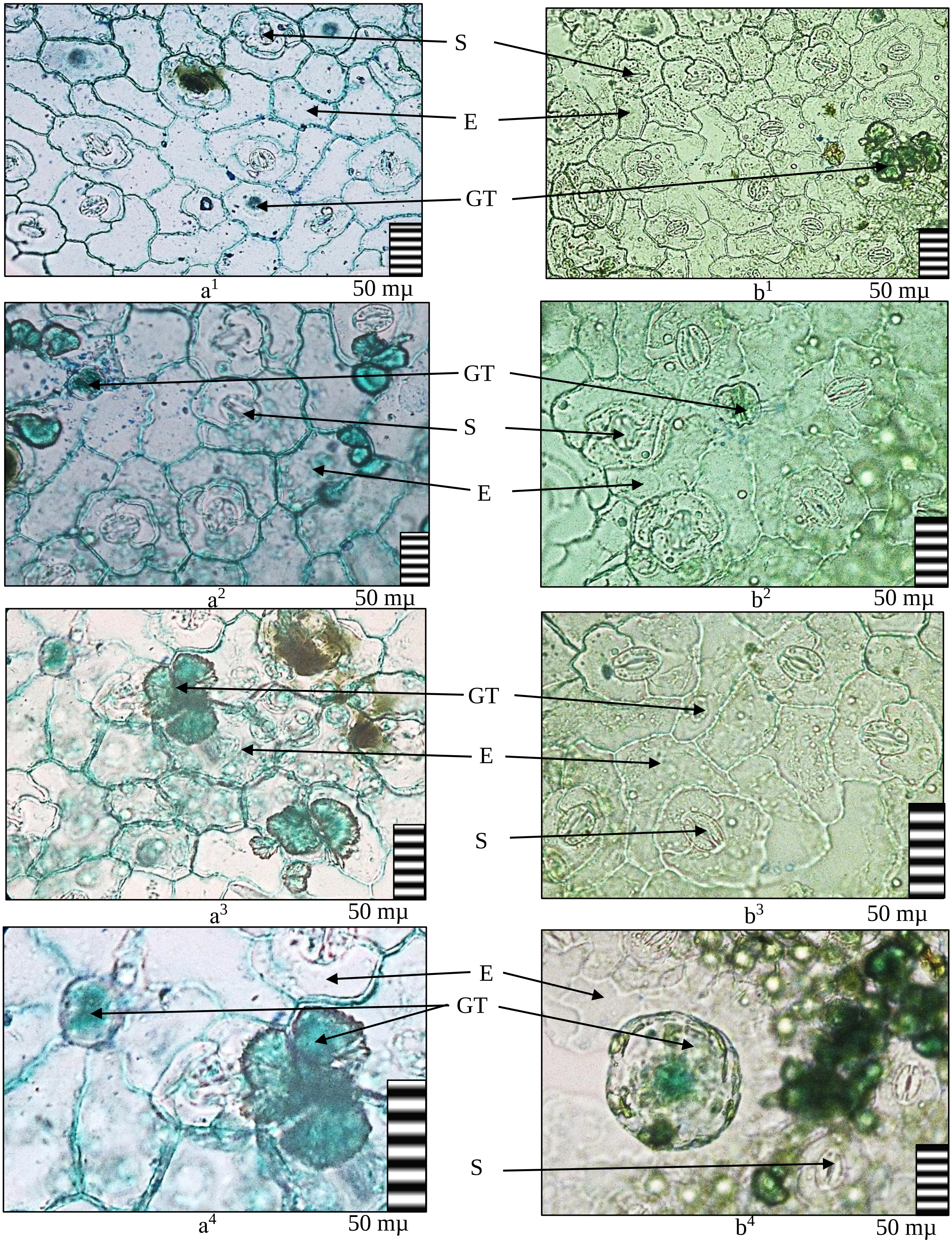

Figure – 2. Anatomical structure of the leaf epidermis of *Hyssopus officinalis* in Tashkent (a^1-a^4) and Jizzakh (b^1-b^4) conditions:
a^1-a^4 – adaxial epidermis (Tashkent Botanical Garden); b^1-b^4– adaxial epidermis.
Legend: GT - glandular trichomes, S - stomata, E - epidermis.

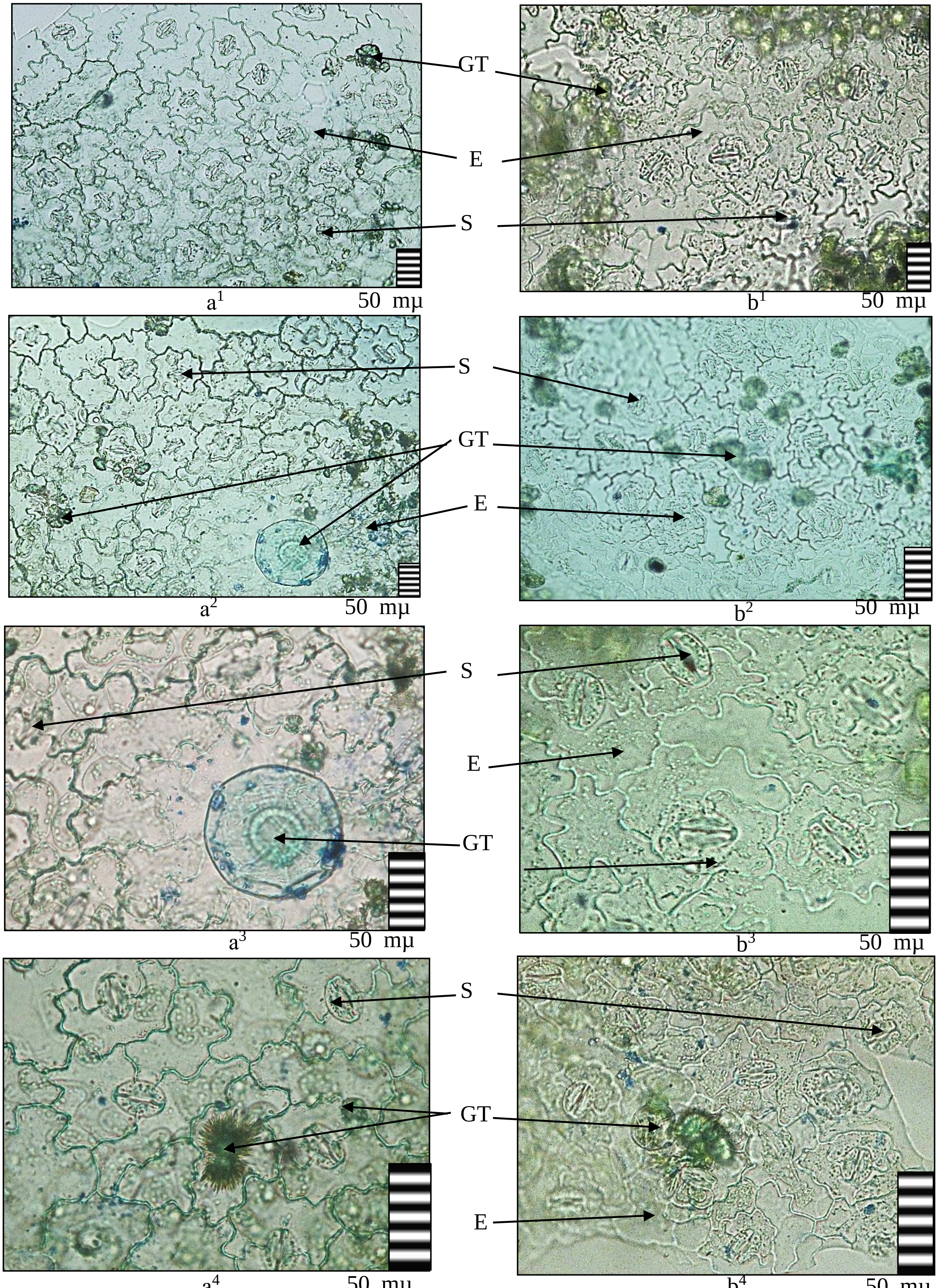

Figure – 3. Anatomical structure of the leaf epidermis of *Hyssopus officinalis* in Tashkent (a^1-a^4) and Jizzakh (b^1-b^4) conditions:

a1-a4 – adaxial epidermis (Tashkent Botanical Garden); b1-b4– adaxial epidermis.

Legend: GT - glandular trichomes, S - stomata, E - epidermis.

The epidermis consists of a number of oval cells, and in the conditions of the Botanical Garden, the cell wall of the epidermis is a thin-walled cuticle, and in the conditions of Jizzakh, the cuticle is thick-walled. The leaf has a without Kranz cell-free mesophyll; between the adaxial and abaxial cells of the epidermis there is an assimilation tissue consisting of columnar and porous cells. In the conditions of the botanical garden, the cells of the palisade parenchyma are large, not numerous - arranged in two rows. And in the conditions of Jizzakh, there are numerous palisade cells - three rows, chlorophyll grains are larger compared to the conditions of the Botanical Garden. These cells are located between the adaxial epidermal and spongy cells (Figures - 4, 5).

Spongy parenchymal cells with chlorophyll granules are located between the abaxial epidermis and palisade cells. In the conditions of the botanical garden, porous cells are large, small, consist of 3-4 rows of round-oval isodermal cells. In Jizzakh conditions spongy cells are small, numerous, consist of 5-6 rows of round-oval cells. There are also intercellular spaces between these cells. Under both conditions, the presence of drops of essential oil in the palisade and spongy cells of the leaf mesophyll was determined. Especially in the conditions of Jizzakh, it was found that the abundance of essential oils in the assimilation tissues of the leaf was more concentrated than in the Botanical Garden. Also in the abaxial epidermis of the leaf there are multicellular glandular trichomes. Among the assimilation tissues there are numerous lateral bundles, consisting of 3-4 xylems of small diameter and phloem (Figures - 4, 5).

The main leaf vein protrudes towards the abaxial epidermis, and its main part consists of thin-walled round-oval parenchyma cells. In its central part there is 1 vascular bundle of a closed collateral type, consisting of phloem and xylem. Xylem cells are thin and thick-walled, elongated, and their tubes are spiral-shaped. In the conditions of the Botanical garden, xylem cells are large, thin-walled, and in Jizzakh conditions, xylem cells are small, thick-walled (Figures - 4, 5).

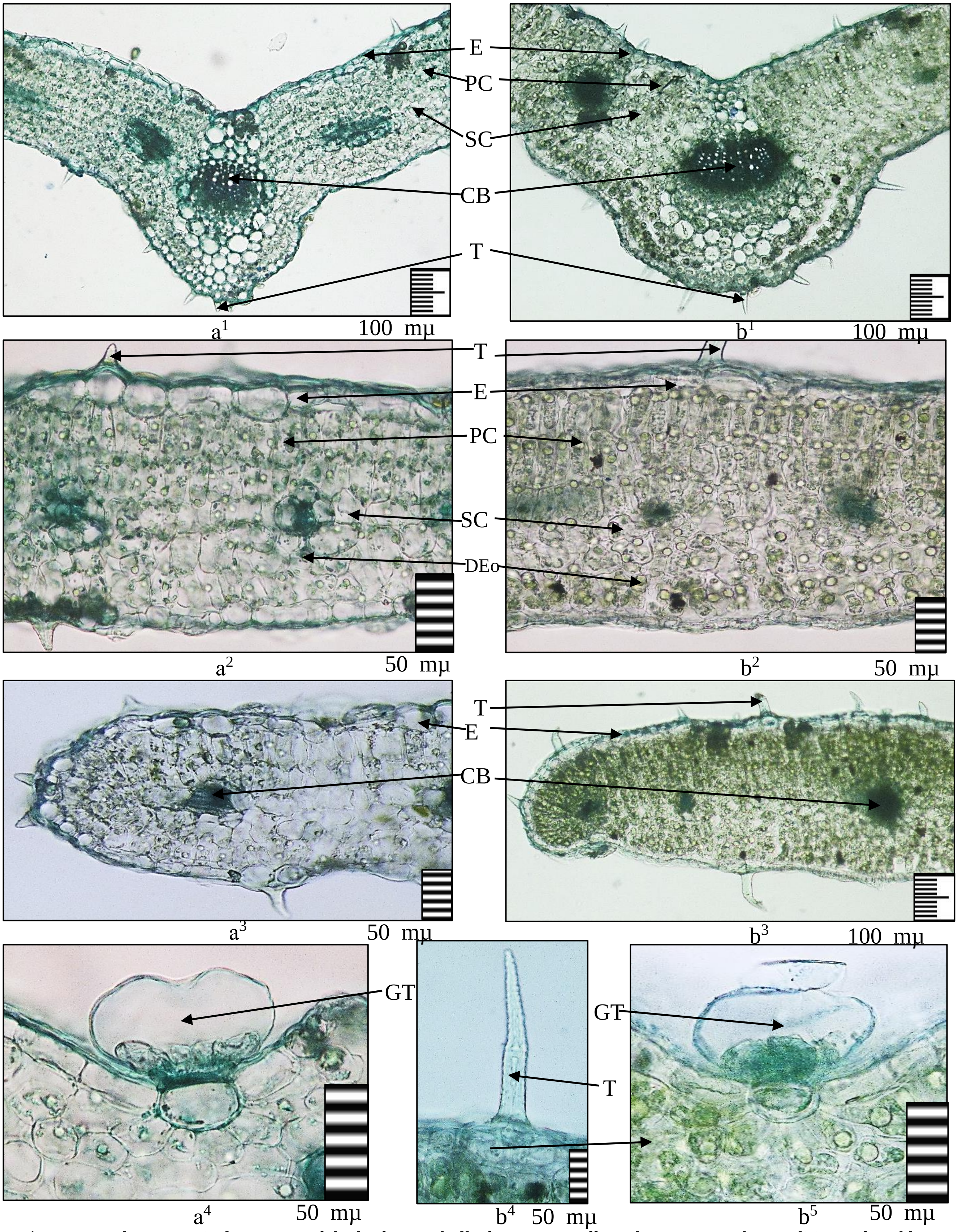

Figure – 4. The anatomical structure of the leaf mesophyll of *Hyssopus officinalis* species in the conditions of Tashkent (a^1-a^4) and Jizzakh (b^1-b^5):

a^1-a^3, b^1-b^3 - detail of the leaf mesophyll; (a^4, b^4) multicellular glandular trichome; b^4 - simple unicellular trichome.

Legend: GT - glandular trichomes, S - leaf stomata, T - trichome, PC - palisade cell, E - epidermis, DEo - drops of essential oil, VB - vascular bundles, SC - spongy cell.

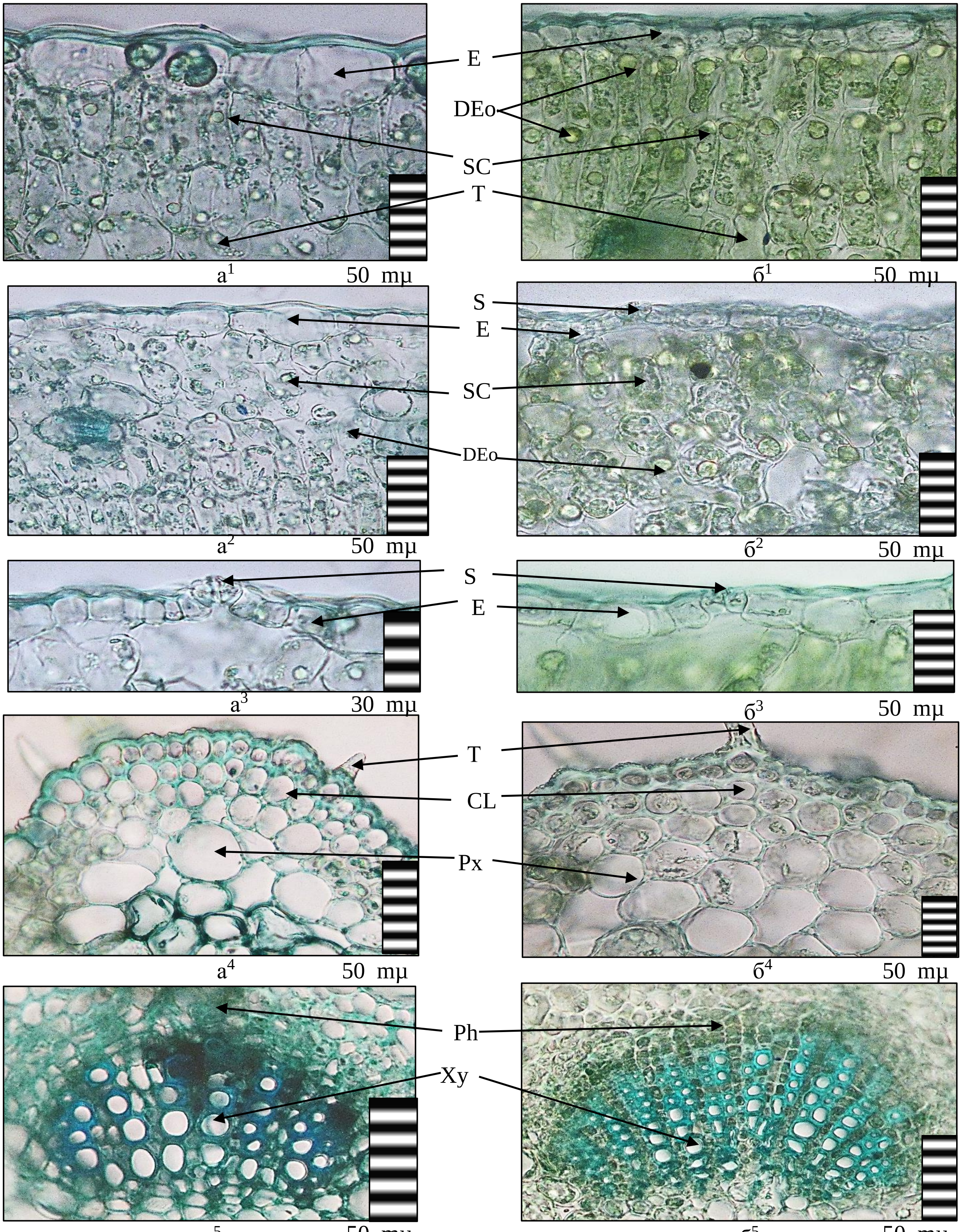

Figure - 5. Anatomical structure of the leaf mesophyll of Hyssopus officinalis species in the conditions of Tashkent (a^1-a^4) and Jizzakh (b^1-b^5):

a1-b1 – palisade cells in a leaf; a2-b2 - spongy cells in the leaf; a3-b3 - unsubmerged stomata; a4-b4 - collenchyma and parenchyma cells; a5-b5 - vascular bundles. Legend: GT - glandular trichomes, S - stomata, CL - collenchyma, Xy - xylem, T - trichome, P - palisade cell, Ph - phloem, E - epidermis, DEo - drops of essential oil, VB - vascular bundles, SC - spongy cells.

In the main vein of the leaf, epidermal cells are single-row, round-oval in shape, under which there are 2-3 rows of charcoal collenchyma cells. Beneath the collenchyma cells are numerous parenchyma cells. Under the conditions of the Botanical Garden, in the main vein of the leaf, the parenchymal cells are thin-walled; in species from the Jizzakh region, the parenchymal cells are thick-walled and round-oval, among which there are hydrocytic cells (Fig. 5).

It has been established that the anatomical structure of the base of the stem of Hyssopus officinalis is round in cross section and has a non-bundle type structure. The diameter of the stem is the largest noted in the conditions of the Botanical Garden and relatively thinner in the conditions of the Jizzakh region (Fig. 6). The energy of the stem of plants is characterized by their strong lignification and the early development of the secondary integumentary tissue. In both cases, pod cells form in the outer part of the periderm of the stem, while living cells form the phelloderm in the inner part. The cells of the pod (cork) are densely pitted, dark brown, densely spaced. Under the periderm there is a secondary parenchyma of the cortex, consisting of 8-10 rows of round-oval cells. Where the edge of the central cylinder joins the primary cortex, there are lignified, thick-walled cells separated by parenchyma - primary bast fibers. In the conditions of the botanical garden, the number of bast fibers in the parenchyma of the bark (3-4), and in the conditions of the Jizzakh region - their abundance, is explained by the adaptation of these species to dry conditions, protection and support. stem tissues and cells from various environmental influences (Fig. 6). Soft lubricating fibers (phloem) are located in the bark of the stem in the form of a ring. The cambial zone in the stem consists of several rows of cells elongated in the tangential direction. It has been established that the woody parenchyma of the stem of a scattered vascular type, the primary conductive tissue, is preserved in the stem and is located near the core of the stem. Secondary conductive tissues in the stem form a round flat cylinder. It was established that cells of sclerenchyma and sclerosed parenchyma are located between the conducting bundles. The cells of the radial beam were elongated, homogeneous and full of tannin. The cells formed during the division of the cambial zone change the anatomical structure of the stem over time. Annually, secondary xylem (wood) is formed from it (Fig. 6-7).

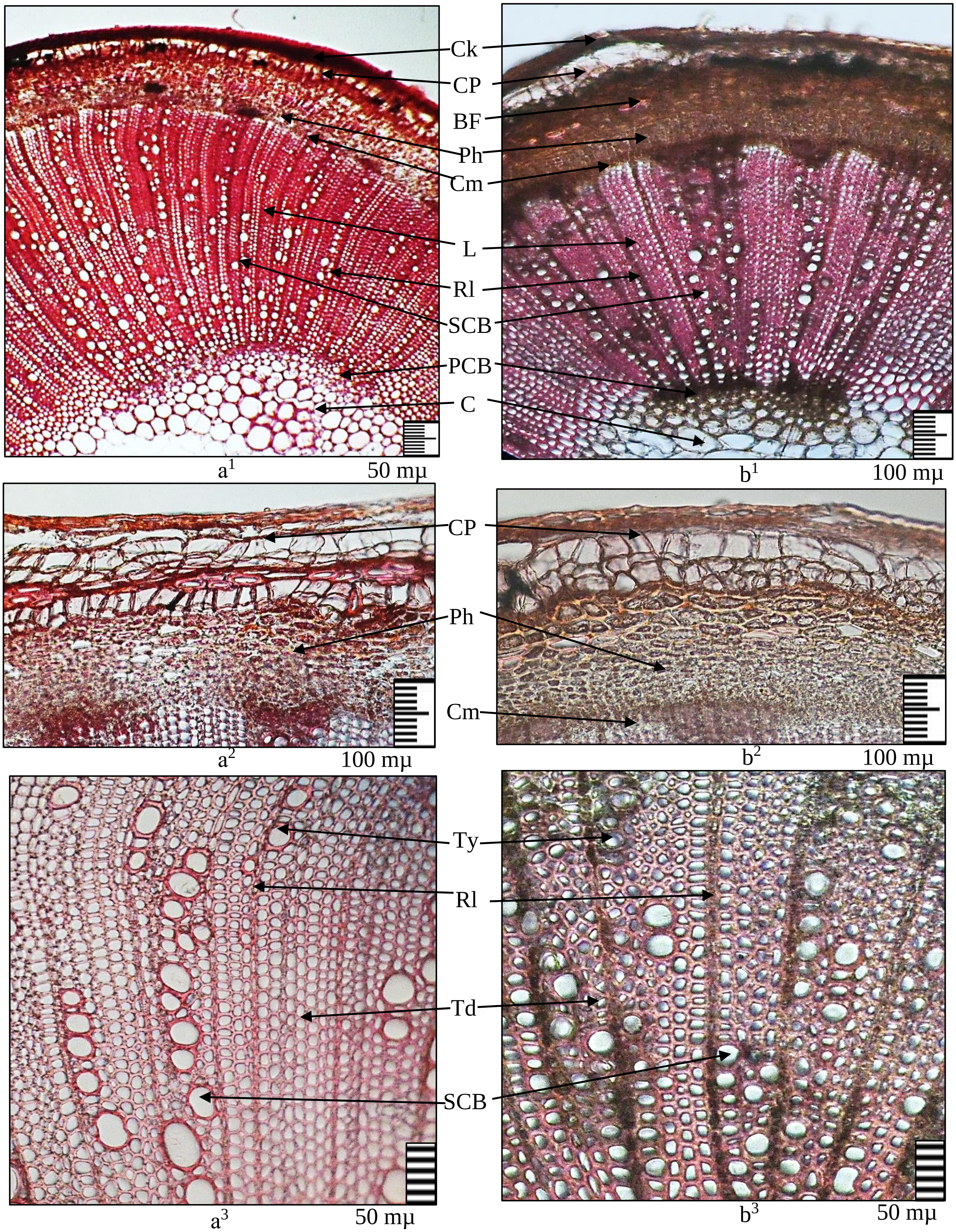

Figure - 6. Anatomical structure of the stem of Hyssopus officinalis species in the conditions of Tashkent (a^1-a^3) and Jizzakh (b^1-b^3):

a^1-b^1 - stem detail; a^2-b^2 - cortex parenchyma; a^3-b^3 - woody part of the libriform stem and secondary vascular bundles.
Legend: PCB - primary conductive bundle, SCB - secondary conductive bundle, Cm - cambium, L - libriform, BF - basted fibers, Ck - cork, CP – cortex parenchyma, Ph - phloem, Td - tracheid, Ty - trachea, Rl - radial (woody rays), C - core.

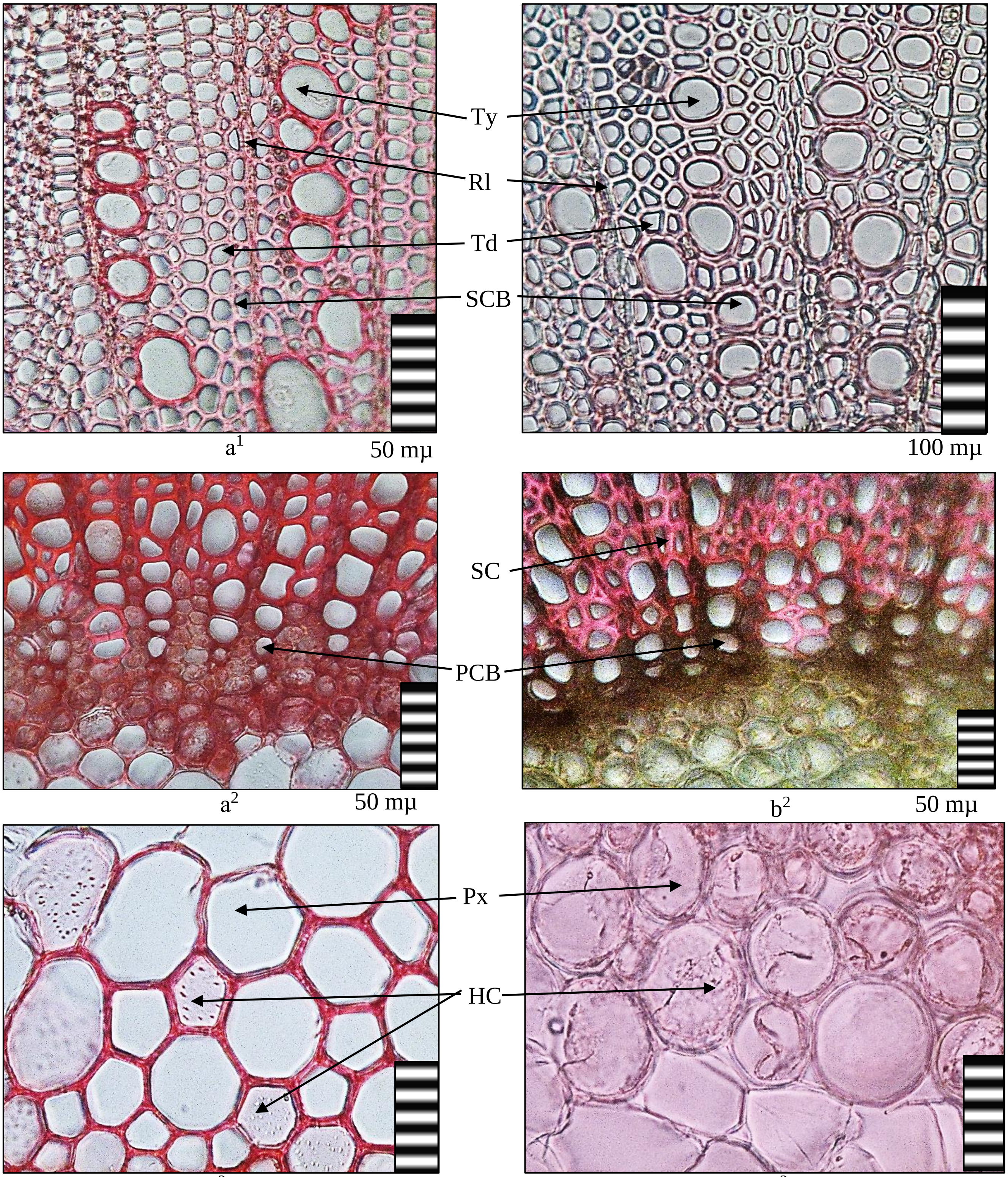

Figure - 7. The anatomical structure of the stem of *Hyssopus officinalis* species in the conditions of Tashkent (a^1-a^3) and Jizzakh (b^1-b^3):

a^1-b^1 - woody part of the stem libriform and secondary vascular bundles;
a^2-b^2 - primary conducting bundles; a^3-b^3 - core: parenchyma and hydrocytic cells. **Legend:** PCB - primary conductive bundle, SCB - secondary conductive bundle, Td - tracheid, Ty - trachea, Rl - radial (woody rays), Px - parenchyma cells, SK - sclerenchyma, GD - hydrocytic cells.

In the core part of the stem, thin-walled parenchyma cells have a round-oval shape, including hydrocytic cells. The central part of the stem takes up less space in the stem. The tubes in the secondary xylem - trachea in the conditions of the Botanical Garden turned out to be larger, smaller and thinner, and in the conditions of the Jizzakh region - the secondary xylem is small, numerous and thick-walled (Fig. 6-7).

It was established that cells of sclerenchyma and sclerosed parenchyma are located between the conducting bundles. It has been established that the elongation of cells of the radial beam, consisting of a number of heterogeneous cells, is filled with tannin. Stem diameter, its ratio to stem diameter, and cell size vary from top to bottom depending on plant habitus and water storage function. Due to the size of the habitus of Hyssopus officinalis growing in the Tashkent Botanical Garden, the diameter of the core part of the stem was determined to be wider. Due to the relatively small habit of the plant, the diameter of the core part of the stem was determined to be narrower in species growing under Jizziax conditions. Also, the presence of hydrocytic cells was noted in the cells of the core parenchyma (Fig. 6-7).

Conclusion

In conclusion, the growth and development of Hyssopus officinalis L. in the first vegetation year in Tashkent conditions and from the second vegetation year in Tashkent and Jizzakh conditions were studied. For the first time, it was found that the Hyssopus officinalis species goes through all phases completely in the second vegetation year under the conditions of introduction - Jizzakh. The growth and development of Hyssopus officinalis species introduced to Tashkent Botanical Garden and Jizzakh conditions, the beginning and end of phenological phases were studied by comparison of air temperature, relative air humidity and soil distribution. Also, for the first time in the conditions of the Tashkent Botanical Garden and Jizzakh, diagnostic signs specific to the species were determined based on the study of the anatomical structure of the assimilative and shoot organs of the species Hyssopus officinalis. Also, on the basis of the comparative analysis of the anatomical signs in the vegetative organs, the structural and adaptive signs characteristic of the conditions of Tashkent and Jizzakh were determined. It was found that mesomorphic characters predominate under the conditions of the Tashkent Botanical Garden, and xeromorphic characters predominate under the conditions of Jizzakh. Also, the localization of biologically active substances was determined in the assimilating organs of the studied species, that is, in palisade and sponys cells. In this species, it was found that there is no internal separating tissue, but biologically active substances are secreted in the assimilative organs due to multicellular glandular trichomes. The identified diagnostic characters are considered permanent taxonomic characters and are used in systematics and in the process of identification of raw materials of the species.

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