

Morphological and Anatomical Characterization of Genotypes of *Origanum vulgare* L. in Uttarakhand

Research Article

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Abstract

Badri tulsi (*Origanum vulgare* L.) is a spice, medicinal and aromatic perennial herb belongs to Lamiaceae family. This plant possesses diverse biological potential due to variability in oil yield related traits. The present study has been conducted on morphological and anatomical characterisation of *Origanum vulgare* L. germplasm collected from Uttarakhand region. The present studies revealed that plants were generally perennial with erect type of habit in most cases whereas semi erect habit were reported in 4 germplasm (Ov-4, Ov-6, Ov-11, Ov-12) collected from Champawat, Chamoli, Dehradun, Joshimath districts respectively. Minimum plant height was reported in Ov-14 germplasm (24.8±1.42cm) collected from Badrinath whereas maximum plant height was observed in Ov-12 (86±1.69cm) germplasm collected from Joshimath district. On leaf length characterization, the minimum leaf length was found in Ov-9 (0.65±0.03cm) while maximum leaf length was reported in germplasm Ov-13 (2.3±0.14cm). In anatomical investigation, transverse section of stem was quadrangular and outer layer was covered with cuticle followed by epidermis with two types of trichome - simple and glandular. Maximum number of trichome on the stem was observed in Ov-11 germplasm and minimum were in Ov-7 germplasm. In transverse section, leaves were covered with cuticle followed by single layer of upper and lower epidermis. Thick cuticle was present in Ov-1, Ov-2 and Ov-12 accessions. Epidermis consists of uniseriate, multicellular long simple and glandular trichome. Diacytic type of stomata was reported in both the surfaces of leaves in all studied germplasm. The findings of the present studies revealed that morphological and anatomical characterization could be used as tools to distinguish the variability among the germplasm of *Origanum vulgare* L. collected from different regions of Uttarakhand, India. Thus, these studies will be useful in future for genetic enhancement and development of varieties with high biomass yield, and desirable agronomic characters with high essential oil content in respect of the conservation of bio resources.

Keywords: *Origanum vulgare* L.; Germplasm; Morphological characters; Anatomical features; Glandular trichome, Essential oil; Conservation

Introduction

Badri tulsi (*Origanum vulgare* L.) is a spice, medicinal and aromatic perennial herb belongs to Lamiaceae family. The plant is native to Europe and north of the African continent [1]. In India, *Origanum vulgare* L. is grown in sub temperate and temperate region especially in Himalayan region.

Badri tulsi is an extremely variable and polymorphic species due

to cross pollination and plasticity. Plants bear terminal corymbose cyme with gynodioecious and male sterility condition having white or purple flowers [2,3]. Plants with a higher essential oil content (*Origanum vulgare* subsp. *hirtum*, *Origanum vulgare* subsp. *glandulosum* and *Origanum vulgare* subsp. *gracile*) species are characterized by the presence of carvacrol, thymol, p-cymene, and γ -terpinene, while the minor constituents are sabinene, linalool, borneol, and sesquiterpenes [4]. The essential oil composition

varies according to environmental conditions, geographical area, harvesting time, and stage of plant maturity [5]. The essential oil of *Origanum vulgare* mostly possess anti-bacterial, anti-viral, anti-septic, anti-microbial, anti-oxidant, anti-fungal, anti-bacterial, anti-coagulant, energetic action, flavouring properties [6]. Traditionally, plant possess several uses like indigestions, diarrhoea, cough, remedy against pruritus, headaches, depression, stomachic, carminative, expectorant, and emmenagogue, respiratory and digestive disorders and ointments to treat wounds [5,7-9]. Besides this, *Origanum* subspecies are widely used in pharmaceutical industry and also serve as a food, preservative and flavouring, cosmetic ingredient and most importantly as a culinary herb [10]. From ancient times in Uttarakhand, India, Badri tulsi serve as prasaad to Badrinath temple as replica of Maa Lakshmi, who is the half dhangini of lord Narayan. According to the literature review, badri tulsi has an amazing ability to combat climate change as it can absorb 12 percent more carbon than ordinary basil and other plants. In Badrinath area more than 1500 people get employment from Badri tulsi, which is the indication of overexploitation of it in hilly regions of Uttarakhand, due to which it can become threat in future.

Traditionally, plant taxonomy dependent on morphological and microscopic characterization of plants. In recent years, morphological and anatomical characterization of Lamiaceae family members is useful for determining the productivity of essential oil along with taxonomical characterization. In this regard, Several studies have been conducted and reported on morphological, microscopic and oil yield characterization of Lamiaceae members. The variability among the populations of *O. vulgare* L. with regard to morphological, histo-chemical characters and composition of essential oil which were basically confined to European countries whereas in India less contribution in these aspects [11-19].

Therefore, the present study was conducted to evaluate variability among the genotypes of *O. vulgare* L. collected from different geographical areas of Uttarakhand on the basis of morphological and anatomical features for enhancing the oil productivity in respect to ex-situ conservation.

Materials & Methods

Study area

The present investigation was carried out at Botany Laboratory in Department of Botany, School of Basic and Applied Sciences and at Agriculture field of School of Agricultural Sciences, Shri Guru Ram Rai University, Patel Nagar, Dehradun, Uttarakhand. The state of Uttarakhand, 27th State of republic of India lies between 28°53'24" and 31°27'50" N latitudes and between 77°34'27" and 81°02'22" E longitude. The state covers an area of 53,483 Km², of which 51,000 Km² comes under the Himalayan region. The state has 13 districts. One of the district and capital of Uttarakhand, Dehradun is nested in the mountain ranges of the Himalaya, is one of the oldest cities of India. Dehradun is situated at latitude 30°31' N and longitude 78°03'21" E, covering an area of 300 sq. Km with an elevation of 650 m above the sea level. Dehradun district is situated in the north-west corner of the state. The average annual rain fall of 2073.mm in the city. The district bordered by the Himalayas in the north, Rajaji range

of the Shivalik hills and lesser Himalayas to the south, the river Ganga to east, and the Yamuna river to the west.

Collection and sowing of samples

A total 14 germplasm of *O. vulgare* with their seeds randomly collected from the natural habitat of different locations of Uttarakhand and from Centre for Aromatic Plants, Selaqui Dehradun, during September to November, 2020. Some germplasm collected from National Bureau of Plant Genetic Resources, Nainital, Uttarakhand. The collected plant material identified with the help of available literature. Raised beds (1mx1m) were prepared and seeds were sown to raise the seedlings of the collected germplasm. The seeds of *Origanum vulgare* were sown in the month of November 2020. Agriculture practices such as irrigation, fertilizer and weeding as per requirement were followed for proper growth of the plants.

Evaluation and collection of morphological data

Morphological characterization has been conducted among the collected germplasm during vegetative and flowering stage on qualitative and quantitative parameters such as plant height, length of third internode from the ground, stem girth, leaf length, leaf breadth, inflorescence length, number of primary and secondary branches, length of petiole, shape of leaf, leaf arrangement, shape of leaf apex and leaf margin.

Anatomical study

Anatomical investigations were carried out on the cross-sections of stems, petiole and leaves. The fresh plant parts preserved in alcoholic acetic solutions which was prepared by the mixing of 50% alcohol, 40% formalin, 2% acetic acid. The preserved plant material was subjected for microscopic studies. All sections were prepared manually and Preserved as well as fresh material was used for anatomical characterization by double staining (safranin and fast green). The transverse section of stem, petiole and leaves were dehydrated in 30%, 50%, 70% and 95% solution of ethanol. For double staining safranin and fast green was used. Double staining was done so that the living tissues can be differentiated from the dead one and at last this double stained section was fixed in the slide by the use of Canada balsam and the photographs of the sections were taken for detail cellular characterization.

Result & Discussion

Badri tulsi is a multipurpose aromatic perennial herb. The main constituents of essential oil of *Origanum* were carvacrol and/or thymol. Oil possesses various biological activities like anti-bacterial, anti-viral, anti-septic, anti-microbial, anti-oxidant etc. In view of wide pharmaceutical and traditional values, the present study has been conducted for morphological and anatomical characterization of genotypes of *Origanum vulgare* L. collected from different locations of Uttarakhand state in India for its conservation. The findings of the present studies are as follows:

Germplasm characterisation on the basis of seed germination and floral initiation:

The collected seeds of *Origanum vulgare* L. germplasm was sown in the month of November, 2020. Initiation of seed germination were

reported in January 2021. The rate of seed germination varies from 48 ± 0.47 to 75 ± 0.47 days. Late Germination of seeds were reported has taken 75 ± 0.47 days in Ov-11 germplasm (75 ± 0.47 days which was) collected from Dehradun whereas early the quick germination (48 ± 0.47 days) was reported in Ov-14 germplasm (48 ± 0.47 days) collected from Badrinath. On the basis of floral initiation, the days varied from 78.3 ± 1.78 to 141 ± 1.69 days. Early flower initiation was recorded in Ov-14 (78.3 ± 1.78 days) germplasm collected from Badrinath while late flowering was recorded in Ov-2 germplasm (141 ± 1.69 days) collected from Uttarkashi (Table 1, Figure 1). Similar type of study was conducted by Ojha et al., in 2014 on 33 accession of *Origanum* collected from different localities of Uttarakhand and he observed that floral initiation was varied from 80.17 ± 0.76 days to 141.17 ± 0.44 days [16].

Morphological characterization

On the basis of morphological characterisation in the present study, It was reported that plants were generally perennial with erect type of habit in 10 germplasm whereas semierect habit were reported noticed in 4 germplasm (Ov-4, Ov-6, Ov-11, Ov-12) were collected from Champawat, Chamoli, Dehradun, Joshimath respectively (Table 2).

In the present study, height of plants varied from 24.8 ± 1.42 cm to 86 ± 1.69 cm. Minimum plant height was found in Ov-14 germplasm

(24.8 ± 1.42 cm) collected from Badrinath whereas maximum plant height was observed in Ov-12 (86 ± 1.69 cm) germplasm collected from Joshimath (Table 3). These studies revealed the effect of altitude on the growth of the plant related to the soil and climatic factors varying seasonally and attitudinally. Similar studies on plant height of *Oregano* has been conducted by Sivicka et al., (2019) on 44 germplasm of *O. vulgare* from Latvia [19]. He observed that plant height of *Oregano* varied from 50.1 to 85.0 cm in 2012, from 45.0 to 75.2 cm in 2013, from 30 to 85.9 cm in 2014. In average, it was 68.14 cm in 2012, 59.7 cm in 2013, 74.12 cm in 2014. Ojha et al., (2014) revealed that the plant height varied from 28.17 ± 0.78 cm to 77.51 ± 7.17 cm on 33 accessions from different geographical conditions of Uttarakhand [16]. Olga et al., in 2018 determine the diversity of 14 different *Oregano* and he observed that plant height varied from 52.13 to 88.66 cm [4]. Weglarz et al., in 2020 studied the difference between two subspecies: *O. vulgare* L. subsp. *hirtum* (link) *letsuwart* (Greek oregano) and *O. vulgare* L. subsp. *vulgare* (common *Oregano*) in central Europe, and reported that the plant height in common *Oregano* was $36.11 \text{ cm} \pm 1.93$ while in Greek oregano, it was 26.15 ± 1.86 cm [18]. Greek oregano plants grown in Poland were about 10 cm lower than common oregano plants. For instance, common oregano plant's height ranged from 18 to 59 cm while in Greek oregano, it varies from 67.8 to 79.9 cm [15, 20-23]. Beemnet et al. in 2014 studied the morphological characteristics of *Oregano* (*Origanum vulgare* L.) in Ethiopia and reported that the average plant height were 34.93 to 39.87 cm [21].

In the present study, effect of altitude on the growth of the plant and other morphological character has been studied. On plant height characterization, maximum plant height (46 ± 1.41 to 86 ± 1.69 cm) was observed in between 1000-2000 m altitude whereas the minimum height (24.8 ± 1.42 to 60 ± 1.41) was observed in between 3000-3550 m (Table 3). Similar findings has been reported by Ojha et al. in 2014 that maximum plant height (35 to 77.5 cm) was observed in between 2000-3000 m altitude whereas the minimum plant height (28-31 cm) was observed in between 3000-3550 m [16]. Effect of altitude on the

Table 1: Variation in seed germination and floral initiation of *O. vulgare* L.

Germplasm	Sowing time	Days taken for germination	Days taken for Floral initiation
Ov-1	12-11-2020	67.6 ± 0.72	140 ± 1.18
Ov-2	12-11-2020	65 ± 0.47	141 ± 1.69
Ov-3	14-11-2020	69 ± 0.47	135 ± 1.65
Ov-4	16-11-2020	64.3 ± 0.72	123 ± 1.69
Ov-5	13-11-2020	62 ± 0.47	123 ± 1.69
Ov-6	12-11-2020	61.6 ± 0.72	122.6 ± 1.44
Ov-7	11-11-2020	61.3 ± 0.27	122.3 ± 1.18
Ov-8	10-11-2020	59.6 ± 0.72	122 ± 1.24
Ov-9	9-11-2020	64.3 ± 0.98	122.6 ± 1.18
Ov-10	11-11-2020	64.6 ± 0.72	120 ± 0.94
Ov-11	1-11-2020	75 ± 0.47	131 ± 0.47
Ov-12	9-11-2020	64.6 ± 0.72	133 ± 1.41
Ov-13	16-11-2020	55 ± 0.47	83.6 ± 0.72
Ov-14	22-11-2020	48 ± 0.47	78.3 ± 1.78

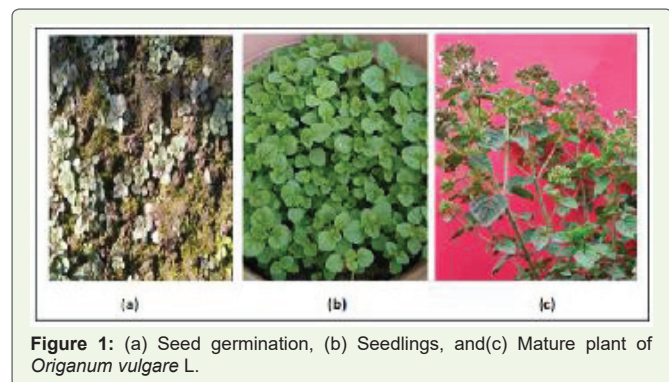


Figure 1: (a) Seed germination, (b) Seedlings, and (c) Mature plant of *Origanum vulgare* L.

Table 2: Details of qualitative characters of accessions in *Origanum vulgare* L. collected from different regions of Uttarakhand.

Accessions	Plant habit	Stem pubescence	Leaf margins	Shape of leaf apex	Colour of petals
Ov-1	Erect	Hairy	Entire	Round	White
Ov-2	Erect	Slightly hairy	Denticulate	Acute	White
Ov-3	Erect	Hairy	Denticulate	Acute	White
Ov-4	Semi-erect	Slightly hairy	Entire	Round	White
Ov-5	Erect	Slightly hairy	Entire	Round	White
Ov-6	Semi erect	Slightly hairy	Entire	Round	White
Ov-7	Erect	Hairy	Entire	Round	White
Ov-8	Erect	Hairy	Entire	Obtuse	White
Ov-9	Erect	Hairy	Entire	Round	White
Ov-10	Semi erect	Hairy	Denticulate	Acute	White
Ov-11	Semi erect	Hairy	Denticulate	Acute	White
Ov-12	Erect	Hairy	Entire	Acute	White
Ov-13	Erect	Hairy	Entire	Round	Pink
Ov-14	Erect	Hairy	Entire	Acute	Light pink

Table 3: Observation on quantitative characters of *Origanum vulgare* L. collected from different regions of Uttarakhand.

Germplasm	Location and Altitude (m)	Plant height (cm)	Stem girth (cm)	Number of primary branches	Length of 3rd internode from the ground (cm)	Length of petiole (cm)	Leaf length (cm)	Leaf breadth (cm)	Inflorescence length (cm)
Ov-1	Bageshwar(1900)	56±0.94	1.4±0.24	7±0.27	12.3±0.27	0.26±0.02	0.76±0.09	0.83±0.13	1.16±0.11
Ov-2	Uttarkashi(1525)	67.6±1.18	2±0.04	8±0.27	9±0.47	0.26±0.02	0.7±0.04	0.7±0.04	1.36±0.07
Ov-3	Tehri(1540)	60±1.88	1.56±0.09	7±0.47	6.6±0.27	0.33±0.02	1.1±0.04	0.08±0.04	1.06±0.05
Ov-4	Champawat(1785)	52±0.94	2.03±0.07	5±0.01	9±0.47	0.33±0.02	0.8±0.04	0.83±0.02	1.2±0.04
Ov-5	Nainital(2153)	54.3±1.90	1.36±0.11	6±0.47	6±0.47	0.2±1.60	1.06±0.05	0.9±0.04	1.33±0.07
Ov-6	Chamoli(2075)	63.1±1.27	1.83±0.05	9±0.47	9±0.47	0.04±0.02	1.86±0.02	1.13±0.05	1.1±0.04
Ov-7	Champawat(1615)	51.6±1.65	1.96±0.09	5±0.27	3.5±0.23	0.13±0.04	0.8±0.04	2.15±1.40	1.1±0.04
Ov-8	Pithoragarh(1600)	55.6±0.98	1.2±0.04	5±0.47	11.3±0.27	0.23±0.02	1.9±0.04	1.06±0.09	1.03±0.07
Ov-9	Champawat(1836)	46±1.41	1.15±0.03	5±0.35	6.33±0.27	0.25±0.03	0.65±0.03	0.45±0.03	1±0.01
Ov-10	Almora(2155)	54±0.94	1.76±0.05	9±0.72	11±0.47	0.26±0.02	1.13±0.07	0.7±0.04	0.96±0.02
Ov-11	Dehradun(430)	35±1.24	1.66±0.07	5±0.47	10.6±0.72	0.36±0.07	0.96±0.02	0.93±0.05	3.13±0.15
Ov-12	Joshimath(1875)	86±1.69	1.76±0.07	4±0.27	12±0.47	0.56±0.09	1.7±0.04	1.5±0.04	1.43±0.07
Ov-13	Tungnath(3680)	60±1.41	1.55±0.03	11±0.70	4.4±0.24	0.9±0.07	2.3±0.14	2.95±0.03	1.95±0.03
Ov-14	Badrinath(3300)	24.8±1.42	0.96±0.02	5±0.47	9±0.47	0.4±0.04	1.96±0.07	1.83±0.13	2.1±0.16

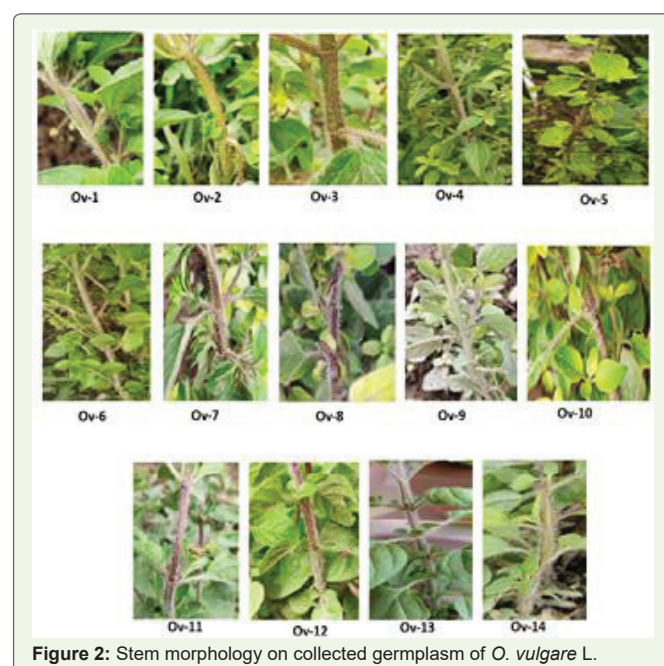
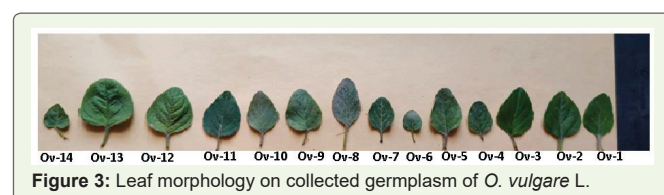
growth of plants was also observed by Krishnan et al. in 2000 reported that variations in the morphological parameters such as plant height related height to the soil and climatic factors varying seasonally and altitudinally [22]. Rajasekaran et al. (1998) observed increase in shoot length with increasing altitude in the plants of same species while grown at an altitudinal gradient [23].

Characterization on the basis of stem colour pigmentation, pigmentation varied from light green (Ov-2, Ov-3, Ov-14, Ov-13), green (Ov-9), light brown (Ov-6, Ov-10, Ov-11, Ov-12) and dark brown (Ov-1, Ov-4, Ov-5, Ov-6, Ov-7, Ov-8) (Figure 2). Similar type of studies has been conducted by Olga et al. in 2018 to evaluate the diversity of 14 different oregano on the basis of colour of stem. Regarding stem pubescence, majority of accessions had slightly hairy stems whereas in two accessions were distinguished by hairy ones (Table 2). Weglarz et al. in 2020 reported that Common Oregano was characterized by erect type of growth and ligneous, slightly hairy stems with stem colour from dark green to red [18]. The number of primary branches ranges from 5±0.27 to 11±0.70. Minimum primary branches were found in Ov-12 (5±0.27) whereas the maximum primary branches were observed in Ov-13 (11±0.70) (Table 3).

In most of collected accessions, petiole length varied from 0.56±0.09cm to 0.04±0.02cm. The minimum petiole length was 0.04±0.02 cm found in Ov-6 germplasm whereas maximum petiole length was found in Ov-12 germplasm (0.56±0.09cm) (Table 3). Stem girth varied from 0.96±0.02cm to 2.03±0.07cm. Minimum stem girth found in germplasm Ov-14 (0.96±0.02cm) whereas maximum stem girth was found in Ov-4 (2.03±0.07) germplasm (Table 3). Most of the stem were covered with white colour hairs. Simple and glandular trichome were found in selected germplasm.

On the basis of leaf morphology, the shape of leaves were ovate in all studied germplasm. The pigmentation of leaves was either green or, dark green. The variation has been reported in leaf apex and margins of leaf. Leaf apex was generally rounded or, and acute, 7 germplasm with round apex and 6 were with acute types has been reported in the present study. Leaf margin was either entire or, denticulate. 10 germplasm found with entire margin and 4 were with denticulate leaf margin and one with obtuse type (Figure 3, Table 2).

Leaf length varied from 0.65±0.03cm to 2.3±0.14cm. The minimum leaf length was found in Ov-9 (0.65±0.03cm) while maximum leaf length was reported in germplasm Ov-13 (2.3±0.14cm). Leaf breadth was varied from 2.95±0.03cm to 0.08±0.04cm. Minimum leaf breadth was reported in germplasm Ov-3 (0.08±0.04cm) whereas maximum leaf breadth was found in germplasm Ov-13 (2.95±0.03cm) (Table 3, Figure 3). Studies on leaf characters has been conducted by Ojha

**Figure 2:** Stem morphology on collected germplasm of *O. vulgare* L.**Figure 3:** Leaf morphology on collected germplasm of *O. vulgare* L.

et al., in 2014 and other scientist. Their studies revealed that leaf length ranges from 1.36 ± 0.12 cm to 2.50 ± 0.07 cm. and the number of primary branches were ranging from 5 to 55. [16]. In most of collected accession, leaves were ovate, broadly ovate, 10-44 mm long and 5-25 mm wide, and phyllotaxy of the leaves were opposite to each other on the stem and the flowers were terminal corymbose cyme [16-18].

In most of germplasm, Inflorescence were terminal or, axillary corymbose. The inflorescence length was varying from 0.96 ± 0.02 cm to 3.13 ± 0.15 cm (Table 3, Figure 4). Flowers were sessile, small, 5-7 mm long, bracts were green and overlapping the calyx. Flowers were generally white and pink in colour (Figure 5). 12 accessions were found with white colour flower whereas 2 accessions with pink flower (Table 2).

Anatomical Characterization

Study

Different accessions of *Origanum vulgare* have been characterised

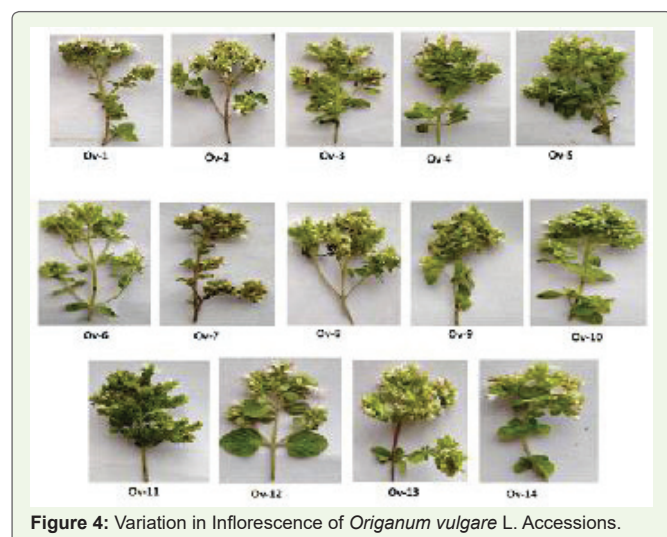


Figure 4: Variation in Inflorescence of *Origanum vulgare* L. Accessions.

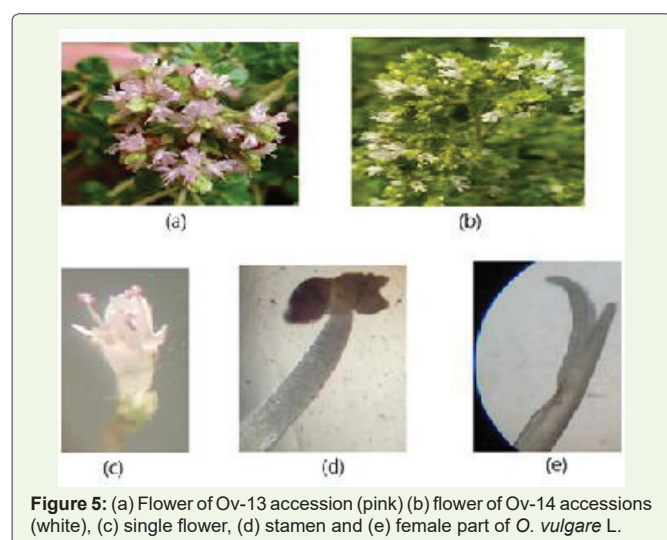


Figure 5: (a) Flower of Ov-13 accession (pink) (b) flower of Ov-14 accessions (white), (c) single flower, (d) stamen and (e) female part of *O. vulgare* L.

on the basis of anatomical character of plant parts such as stem, leaf and petiole as follows:

Stem

The shape of stem was quadrangular which the characteristic feature of Lamiaceae family. Outermost layer was covered with cuticle followed by epidermis with two types of trichome- simple and glandular. Maximum number of trichome on the stem were observed in Ov-11 germplasm and minimum were in Ov-7 germplasm. Epidermis was followed by 5-8 cortical layers generally made up of parenchymatous cells. Thick cortical layer was observed in Ov-12 (6-8 layered) and thin cortical layer found in Ov-14 (3-4 layered) germplasm. Collenchyma cells basically present at the corners of the stem. Well-developed notch with smaller collenchyma cells were present in Ov-7, Ov-1 and Ov-9 germplasm. Single layer of endodermis followed by parenchymatous pericycle layer was observed. The central region of stem consists of vascular bundles i.e. xylem, phloem and followed by pith. The phloem includes sieve-tubes and phloem parenchyma. Cambium was present in distinct patches. The xylem was differently structured; the primary xylem contains vessels placed in radial rows separated by cellulosic parenchyma cells, the secondary xylem, much thicker has vessels dispersed in radial rows too, but separated by libriform fibres. Xylem vessels were arranged in four groups connected with narrow band of xylem tissue. Maximum number of xylem vessels were found in Ov-12 (36 rows) accession and minimum number of xylem vessels were found in Ov-14 (18 rows) germplasm. Central portion i.e. was occupied with parenchymatous cells. Medullary rays made up of parenchymatous cells varied from 18-36 rows. Maximum medullary rays were found in Ov-12 accession (36 rows) whereas minimum number of medullary rays were found in Ov-14 (18 rows) germplasm (Figure 6). In all studied

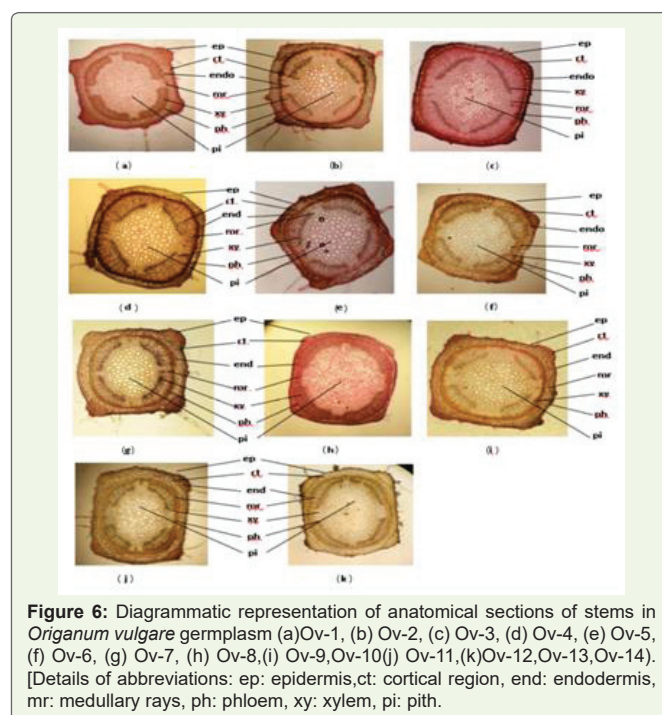


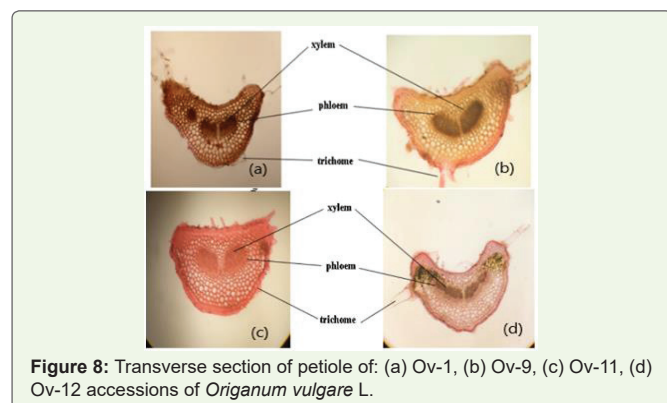
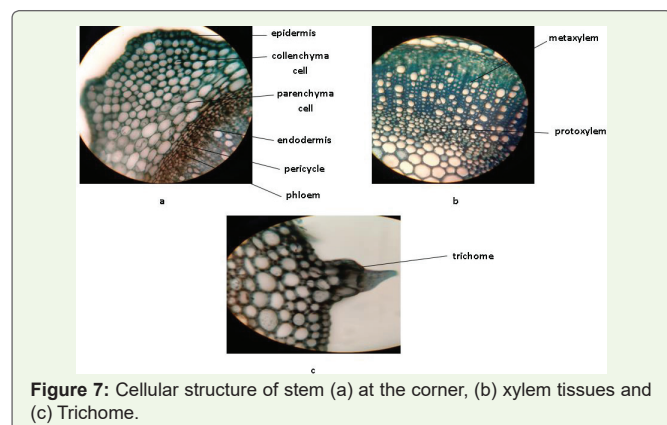
Figure 6: Diagrammatic representation of anatomical sections of stems in *Origanum vulgare* germplasm (a) Ov-1, (b) Ov-2, (c) Ov-3, (d) Ov-4, (e) Ov-5, (f) Ov-6, (g) Ov-7, (h) Ov-8, (i) Ov-9, (j) Ov-10, (k) Ov-12, Ov-13, Ov-14). [Details of abbreviations: ep: epidermis, ct: cortical region, end: endodermis, mr: medullary rays, ph: phloem, xy: xylem, pi: pith.

accessions, vascular bundles were arranged in four different patches and vascular bundle were conjoint collateral, endarch and open type (Figures 6,7). Similar type of study was on *Origanum* plant in Uttarakhand done by Ojha et al., in 2014 [16].

Epidermal structures and stomatal ontogeny of some medicinal plants have been done by many scientists and reported that epidermal and cuticular traits of plants like epidermal cells, size and shape of trichomes and number of vascular bundles could serve as vital tools in solving taxonomic problems in Angiosperms [24- 26].

Petiole

The petiole shape was sulcate with obtuse margins in all the selected accessions. Single layered epidermal cells were present in both upper and lower layer. The surface was covered with cuticle. Thick cuticle was present in Ov-11 and Ov-12 germplasm whereas thin cuticle was present in Ov-1, Ov-9 germplasm. Multi-layered collenchyma cells were present at the corner regions. The vascular bundles were distributed in the parenchyma tissues. Petiole consists of 2-3 vascular bundle patches. Two vascular bundle patches were found in Ov-1, Ov-11 accession, whereas three vascular bundle patches were found in Ov-9 accession with half circle shape. The vascular bundles were arc shaped in Ov-12 accession. Well-developed notch was found in Ov-9 and Ov-12 accession (Figure 8). Similar types of vascular bundles were also reported in *Ocimum* [27]. The shape of the petiole and central vascular bundle has diagnostic value in species characterization in members of Lamiaceae family [28].



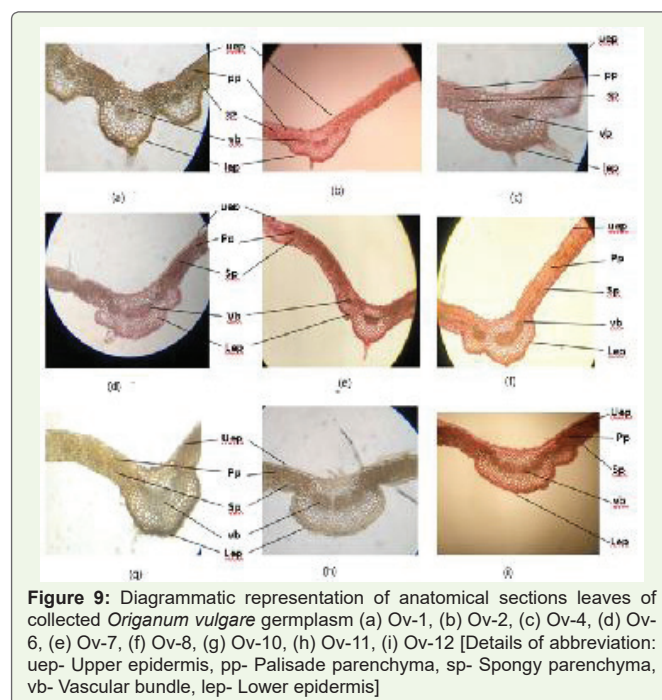
Leaves

In transverse section, leaves were covered with cuticle followed by single layer of upper and lower epidermis. Thick cuticle was present in Ov-1, Ov-2 and Ov-12 accessions. Epidermis consists of uniseriate, multicellular long simple and glandular trichomes. Beneath the upper epidermis, palisade parenchyma and spongy parenchyma were present. Single layered palisade parenchyma was present in Ov-1, Ov-2, Ov-3, Ov-11 accession. 4 to 5 layered spongy parenchyma were present in all the studied accessions. The vascular bundles were present in 2 to 3 different patches. Two vascular bundle patches were found in Ov-1, Ov-4, Ov-3 and three vascular bundle patches were found in Ov-2, Ov-5, Ov-6, Ov-7, Ov-8, Ov-9, Ov-10, Ov-11 and Ov-12 germplasm (Figure 9). Diacytic type of stomata was reported in both the surfaces of leaves in all studied germplasm.

Similar type of studies has been conducted by Ojha et al., (2014). According to his studies on anatomical features of *Oregano*, leaf was dorsiventral, diacytic type of stomata, trichome were simple or covering type and glandular type. The upper and lower epidermal cells were found to be wavy with thin cell wall. Vascular bundle is restricted to the midrib region and comprises of collateral arrangement of xylem and phloem [16].

Trichome

Trichomes are hair like unicellular and multicellular appendages emerge from the surface of the leaves and stem. The role of trichomes is to help in secretion of essential oil, absorption of water, ion exchange, protection etc [29]. In *Origanum* subspecies and in other members of Lamiaceae family, glandular trichome was multicellular epidermal glands responsible for storage of essential oil. Two different types of glands were recognized on the epidermis of *Origanum* species i.e., peltate and capitate types. The glandular trichomes are built of one



basal cell, one stalk cell and a multi-cellular head, which synthesized essential oil after that it transferred to subcuticular area [30,31].

In the present studies on *Origanum vulgare* plants, non-glandular trichome and glandular trichome were found in stem and leaves. Non-glandular trichomes were unbranched, uniseriate, Hooked, Multicellular and generally originated from epidermal cells. Glandular trichomes were unicellular and uniseriate. These trichomes can secrete variety of secondary metabolites. Most of the trichomes present on stem and leaves were with multi cellular base. Glandular trichome were capitate type. Maximum number of non-glandular trichomes and glandular trichomes were found in Ov-11, Ov-12 and Ov-6, Ov-11 accession respectively (Figure 10).

In some genera of Lamiaceae family, trichome morphology is helpful in infrageneric as well as at specific levels classification [31]. Weglaz et al. in 2020 reported that Greek oregano was distinguished by visibly higher number of glandular trichomes on the leaves (up to 4.85 per 1 mm²) followed by higher content of essential oil in the herb in comparison of common oregano [18]. The factors that influence the density and glandular trichomes, and, thus, directly impact the yield of essential oil, are defined both genetically and environmentally. Reduced water and nitrogen supply augment the density of peltate trichomes, while plant density does not affect the number of glandular hairs per leaf surface unit [32]. Kofidis et al., (2003) investigates the structural variations of *Origanum vulgare* leaves during growing season at native plants from different altitude (200 m, 950 m and 1760 m). The glandular hairs are more numerous on the lower leaf surface than on the upper one at 200 m altitude. In the leaves of lowland (200 m) plants, glandular hairs are denser compared with those of the mid and upland (950 m, 1760 m) plants, for both the upper and lower surface, which helps in pollination [32-34].

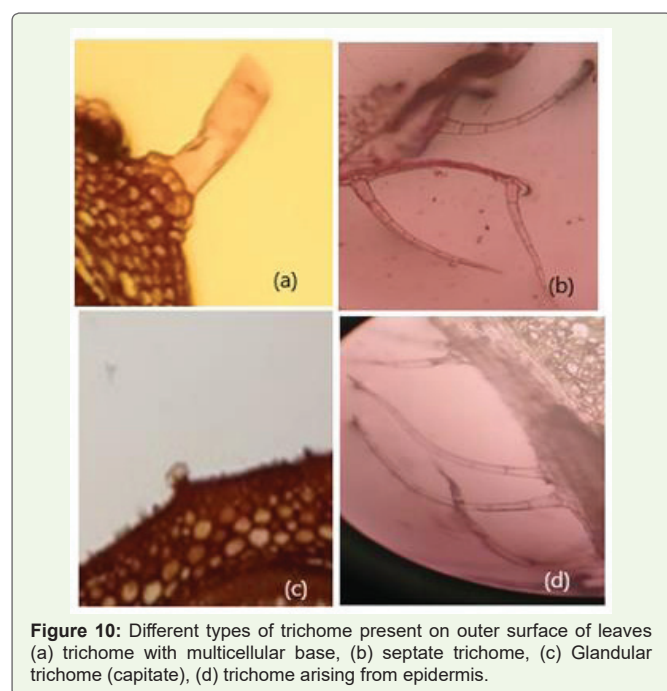


Figure 10: Different types of trichome present on outer surface of leaves (a) trichome with multicellular base, (b) septate trichome, (c) Glandular trichome (capitate), (d) trichome arising from epidermis.

Conclusion

The findings of the present study on morphological and anatomical characterization of different plant parts could be used as taxonomical tools to distinguish the variability among the germplasm of *Origanum vulgare* L. collected from different regions of Uttarakhand, India. Thus, these studies will be useful in future for genetic enhancement and development of varieties with high biomass yield, and desirable agronomic characters with high essential oil content in respect of the conservation of bio resources.

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