



Altitudinal Variations of Essential Oil-Secreting Tube Cells in the Vegetative Organs of *Coriandrum sativum* L.

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Abstract

Modifications in the anatomical structure of plants grown in different conditions have been reported, such as increase in number of palisade mesophyll layers, size and density of stomata. However, these variable responses to environmental factors depend on the physiological and morphological characteristics of the species. In this study, anatomical features on the vegetative organs of *Coriandrum sativum* L. from two areas with different elevations were evaluated with emphasis on essential oil-secreting tube cells (EOT). Light microscopy techniques were employed in examining the transverse sections of roots, stems, and leaves. Anatomically, the *C. sativum* roots, stems, and leaves had the same tissue compositions in both sampled areas. However, the diameter of EOT significantly differed in between two areas. Cross sections of *C. sativum* from La Trinidad, Benguet which represented the area with higher elevation had significantly larger diameter of EOT in all sampled vegetative organs. In both areas, EOT were localized in the cortical region of the roots, below the hypodermal area of the stem and adjacent to its vascular bundles, and within the spongy parenchyma layer of leaves. In roots, EOT cells were randomly arranged within the cortical region, in a ring-pattern in stems following the vascular bundle arrangement, and adjacent to vascular bundles in leaves.

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Introduction

Coriandrum sativum L. is a member of the order - Apiales, under family Apiaceae (Umbelliferae) (Sharma and Sharma, 2012). It is an annual herb that originates from the temperate geographical areas of Europe and Asia. At flowering, the glabrous plant can reach heights within a range of 0.2-1.40 m. Diederichsen (1996) described the stem as erect, green and becomes hollow at maturity. Its leaves are alternately arranged, and the first ones are often gathered in rosette. The blade shape of the leaves is either undivided with three lobes/ tripinnatifid, while the leaves of the nodes following are to a high degree pinnatifid. Thus, the upper leaves are deeply incised (Diederichsen, 1996).

This plant is commonly known as “coriander or cilantro”. When consumed fresh, this plant is referred as cilantro while if used as spice, it is often called coriander (Kofidis *et al.*, 2008). The global economic demand of cilantro has been increasing with the expanding acceptance of dishes and cuisines that add cilantro. It is a medicinal and an aromatic plant used in many cuisines. Traditionally, the leaf of *C. sativum* is used as a garnish for food. However, other vegetative organs such as root and stem and reproductive organs such as flower and fruit are also utilized for they also secrete odors and produce volatile organic compounds.

The oil-accumulating organ or tissue varies between species and no single part of the plant is excluded from the list of organs which can serve as oil stores (Silva, 1995). These compounds are generally used as attractants for species-specific pollinators or to protect plants by repulsing herbivores and pathogens (Caissard, 2004).

The characteristic smell of the plant that can be likened to bugs is due to the aldehyde content of the essential oil (Deng *et al.*, 2003). Indeed, the plant's name is derived from the Greek name for bug, *korion* (Diederichsen, 1996). The essential oils of *C. sativum* contain active compounds, which call the attention because of the variety of biological activities

that they present, such as stimulant, narcotic, antihelminthic, and stomachic. In addition, many studies conducted extractions of *C. sativum* and found out different properties of the herb such as antibacterial (Lo Cantore *et al.*, 2004), antimicrobial (Begnami *et al.*, 2010), anticonvulsant, anti-inflammatory, anxiolytic, anthelmintic, and antioxidant (Melo *et al.*, 2003). Its antimicrobial activity is due to the presence of alcohols: 1-decanol, 2E-decenol, 2Z-dodecenol, and aldehydes extracted from the essential oil of this plant (Begnami *et al.*, 2010). The herb, that is common as culinary ingredient because of its aromatic flavor, is indeed considered to have many medical uses and therapeutic benefits too.

Environmental factors constantly influence the production of essential oils in plants and other secondary metabolites, which also cause phenotypic plasticity as adaptive structures. At different altitudes, atmospheric pressure, warmth index, radiation, humidity, wind speed, and precipitation are some of the factors that alters with the change in altitude and influence anatomy and essential oil production (da Cruz *et al.*, 2014; Luo *et al.*, 2014). In saffron, Fatemeh *et al.* (2011) reported higher concentration of major metabolites and significant differences in leaf anatomy, such as cuticle thickness, diameter of cortex and palisade cells. In addition, anatomical observations of thyme showed spatial increase in root cortex, increase in collenchyma layers of stem, and stem cortex with increase in altitude (Gönüz and Özürgücü, 1999).

Most researchers have done extraction of *C. sativum* essential oils (Zoubiri and Baaliouamer, 2010; Soares *et al.*, 2012; Mandal and Mandal, 2015) and studied its composition but only few studies focused on the cell structural level. This study evaluated the essential oil secreting tube cells in the vegetative organs of *C. sativum* using light microscopy techniques. Location, distribution pattern, and diameter of essential oil secreting tube cells in the roots, stems, and leaves of *C. sativum* were compared in two areas with different elevations.

Materials and methods

Plant Material

Coriandrum sativum L. plants were obtained from commercial farms from two locations: La Trinidad, Benguet ($16^{\circ}26'51.0''\text{N}$ $120^{\circ}34'29.5''\text{E}$) and Bay, Laguna ($14^{\circ}10'45.3''\text{N}$ $121^{\circ}17'09.9''\text{E}$) (Fig. 1). The second site is about 318 km south of the first

site. Samples from La Trinidad, Benguet were grown at high altitude of 1,337 m above sea level. Plants from the second site were cultivated at lower altitude of 8 m above sea level. The vegetative parts of the plant including the leaves, stems and roots were utilized in this experiment.

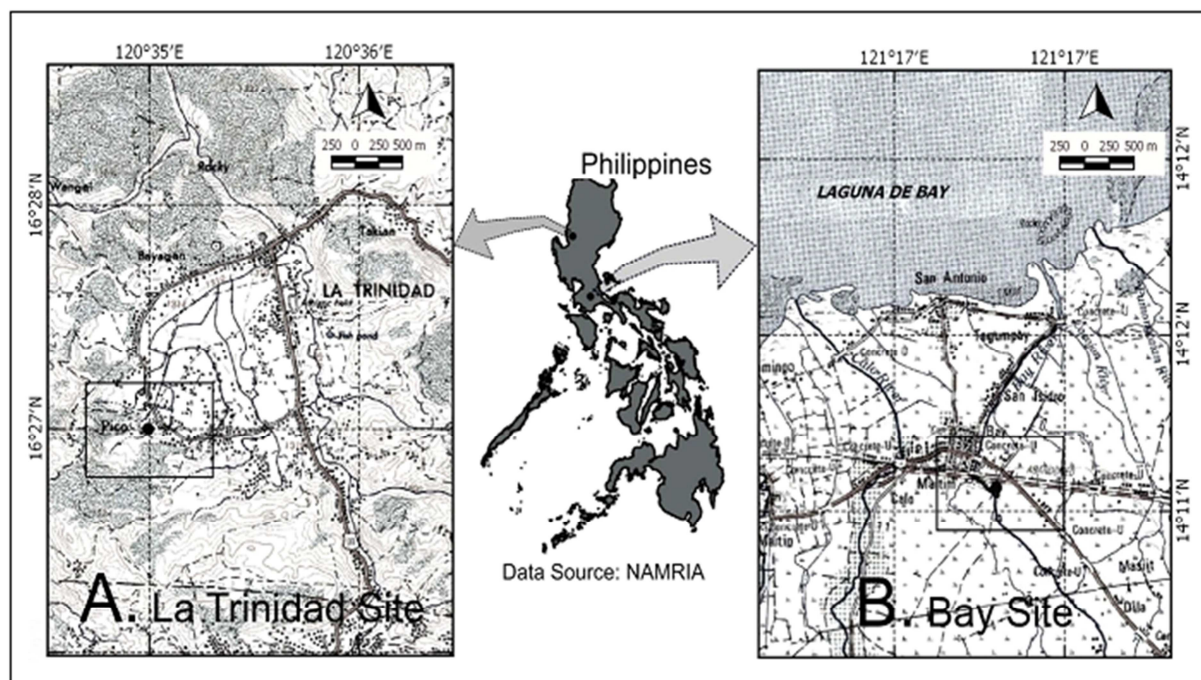


Fig. 1. Location of study sites. A. La Trinidad site-representing higher altitude. B. Bay site-representing lower altitude.

Tissue Preparation and Examination

Samples for anatomical examination and measurements were taken from fresh leaves, stems and roots. Hand-cut sections from each organ, with at least 5 mm thickness were fixed for at least 24 h using the fixative, formalin-acetic acid (FAA). The fixed sections were washed with distilled water and dehydrated using serial concentrations of ethyl alcohol 50%, 70%, 90% and 95% respectively, followed by isopropyl alcohol. For clearing, the segments were subjected to xylene gradually.

Wax embedding was carried out in an oven adjusted at 60°C where the plant sections were transferred every 60 min from a mixture of paraffin wax and xylene (95% paraffin; 5% xylene), into pure paraffin wax and finally into another container of pure paraffin wax. The melted wax, containing the plant

segments was cooled in water, sliced and molded into blocks.

The samples were sectioned transversely using a rotary microtome (Leica RM2135) adjusted at $11\ \mu\text{m}$. Using a brush, the ribbons of sections were collected on glass slides, previously wetted with egg albumin to keep the sections attached to the slides. The slides were left overnight on a hot plate to give maximum expansion of the tissues. Dewaxing was done by immersing the slides with their sections in pure xylene for 20 min. The sections were dehydrated by transferring them into series of ethyl alcohol concentrations 95%, 90%, 70% and 50% respectively (Eltahir and Abuereish, 2010) and washed with distilled water. Staining was done by flooding the slides containing the sections with Safranin O (2% in 50% ethanol) for 30 min.

Then washed with 95% ethyl alcohol and counter-stained with Fast Green (0.05% in 80% acetone) for 10-15 sec.

The slides were washed with 2-propanol and xylene. The slides were mounted using a Canada balsam and covered with coverslip.

The prepared slides were left to dry in an oven adjusted at 60°C for at least 60 min.

The prepared slides were viewed under a microscope (Zeiss AxioPlan2; Olympus DP70) at Histology Laboratory, Plant Breeding, Genetics and Biotechnology Division, International Rice Research Institute (IRRI).

Statistics

Statistical analysis was performed for each parameter with three replications (n=45). Data on the diameter of essential oil-secreting tube cells between organ types and between two locations were tested for significant difference using t-test. Data analysis was performed using STAR 2.0.1.

Results and discussion

Anatomy

Anatomical examination of *C. sativum* roots, leaves and stems showed a typical anatomical pattern found in dicots (Fig. 2). Cross-sections of roots from Bay and La Trinidad exhibited mainly three parts: epidermis, cortex and stele.

Table 1. Comparison on the number of essential oil-secreting tube cells in the vegetative organs of *C. sativum* from Bay, Laguna and La Trinidad, Benguet.

	Bay, Laguna			La Trinidad, Benguet		
	Roots	Stems	Leaves	Roots	Stems	Leaves
No. of EOT cells	8-11	11-20	7-19	—	12-14	6-8

The epidermis forms a single layer of closely packed cells on the outermost region of the root. Below the epidermis is the cortical region that is multilayered. Generally, this region is composed of thin-walled parenchymatous cells with prominent intercellular spaces.

The cells vary in shape from round to oval. The central part of the root is the stele with the prominent vascular strands. The essential oil-secreting tubes (EOT) can be found embedded in the cortical region of the root from Bay, Laguna (Fig.3A-B).

This observation is in line with the findings of earlier studies (Diederichsen, 1996; Morales-Payan, 2004) that the whole plant, even the roots, contain specialized cells that produce essential oils that render the plant its characteristic aroma and flavor.

In another anatomical study on *Chaerophyllum astantiae* and *C. aureum* that are members of the same family Apiaceae (Yilmaz and Tekin, 2013),

secretory ducts are found in the cortical region surrounded by 4-8 glandular cells. However, these cells were not evident in the root samples taken from La Trinidad, Benguet (Fig. 3C-D). This can be attributed to the dilation of the parenchyma cells as it expand or might be due to the formation of aerenchyma.

Transverse section of stem from two areas exhibited the characteristic dicot pattern (Fig. 4). The outermost region of the stem is surrounded by the epidermis. The epidermis is a unicellular layer of barrel-shaped cells and closely positioned to each other. Below the epidermis is the hypodermal area, considered as the outermost region of the cortex. It is multilayered and collenchymatic. They are easily distinguished in having a primary cell wall that is unevenly thickened.

This is mechanical tissue that strengthens the stem, adds its flexibility and elasticity. Because of their function, these cells are often found at the periphery

of stems (Simpson, 2010). Internal to the collenchymatous cells is the essential oil-secreting tube. The middle region of the cortex is parenchymatous. Its shape varies from round to oval with the presence of intercellular spaces. On the inner

region of the cortex, vascular bundles are present arranged in a ring pattern. Essential oil-secreting tubes can also be found adjacent to vascular tissues (Kofidis et. al., 2008). In the center of the stem is the pith composed of large parenchyma cells.

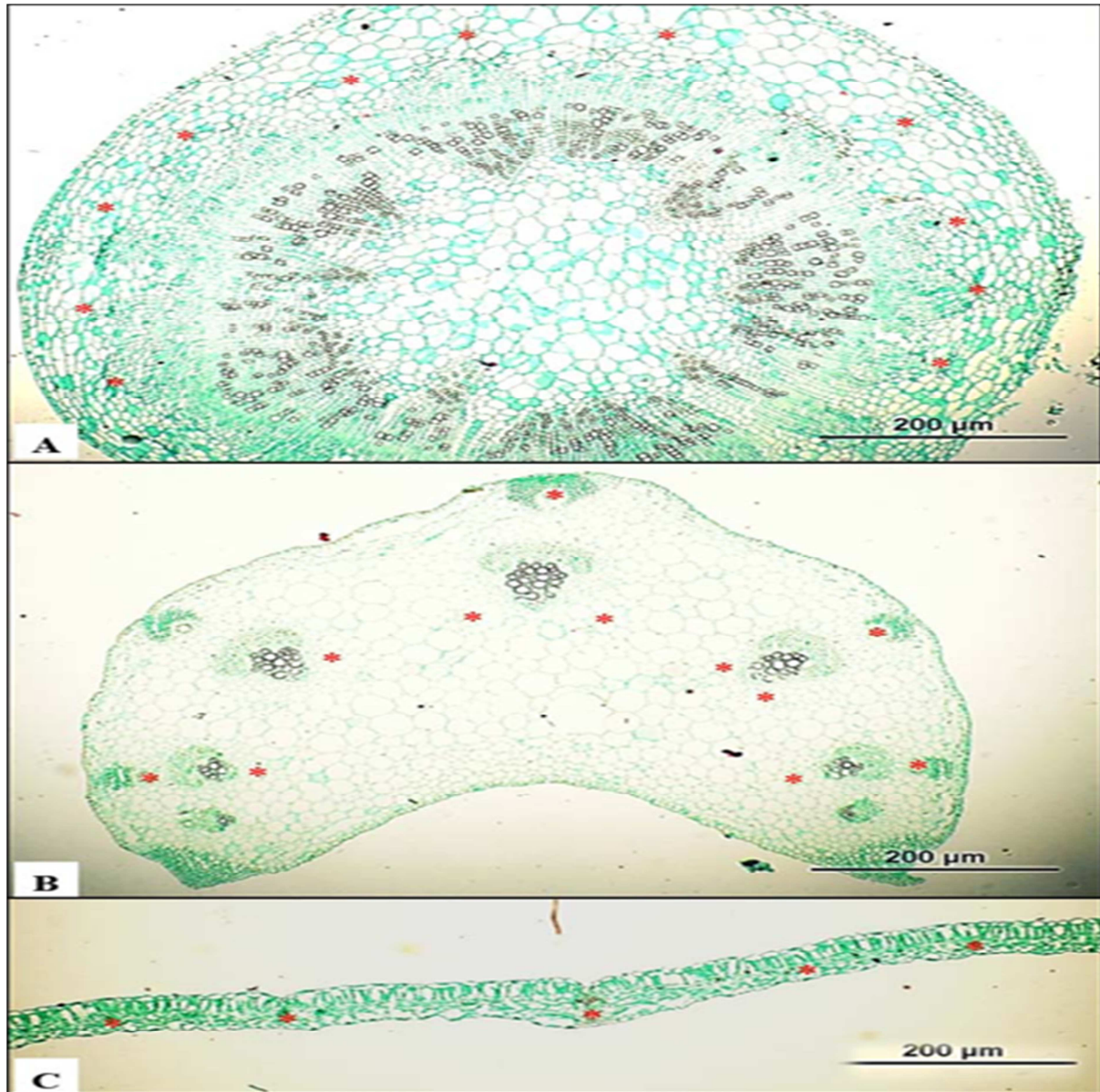


Fig. 2. Cross sections of root (A), stem (B), and leaf (C) of *C. sativum* under light microscope. *-essential oil-secreting tube cell.

Examination of cross sections from leaves of both area showed the same dicot arrangement (Fig. 5). Both surfaces of the blade are bounded by the upper and lower epidermis. The stomata present in both epidermis is anisocytic, with more frequent stomata in the lower epidermis. The mesophyll layer lies in between the two epidermis. This layer is subdivided

into two sublayers: palisade and spongy mesophyll layers. Below the upper epidermis are the columnar, pillar-shaped cells of the palisade parenchyma layer. The one in contact with the lower epidermis is the spongy mesophyll layer that is distinguishable by the presence of air spaces. Within the spongy parenchyma layer are the variously sized EOT (Fig.

5B and 5D). Our findings are in line with earlier studies on the presence of essential-oil secreting tubes in *C. sativum* leaves (Kofidis et al., 2008).

The pattern of spatial arrangement of secretory cells in Asteraceae is mostly associated with the vascular system. In *Arnica* species, anatomical sections of leaves, rhizomes, and roots showed a well-developed secretory system that is situated along the vascular system (Kromer et al., 2016). Similarly, in the studied

C. sativum, EOT cells were symmetrically placed along vascular bundles. However, EOT cells are found also in many other places such as root cortex, below the hypodermis in stem, and mesophyll in leaves. In *Matricaria* leaves, localization of oil secretory structures in the mesophyll area is also recognized (Andreucci et al., 2008). Similar to EOT cells in the roots of *C. sativum*, the essential oil secretory structures are localized in the cortical region (Pljevljakušić et al., 2012).

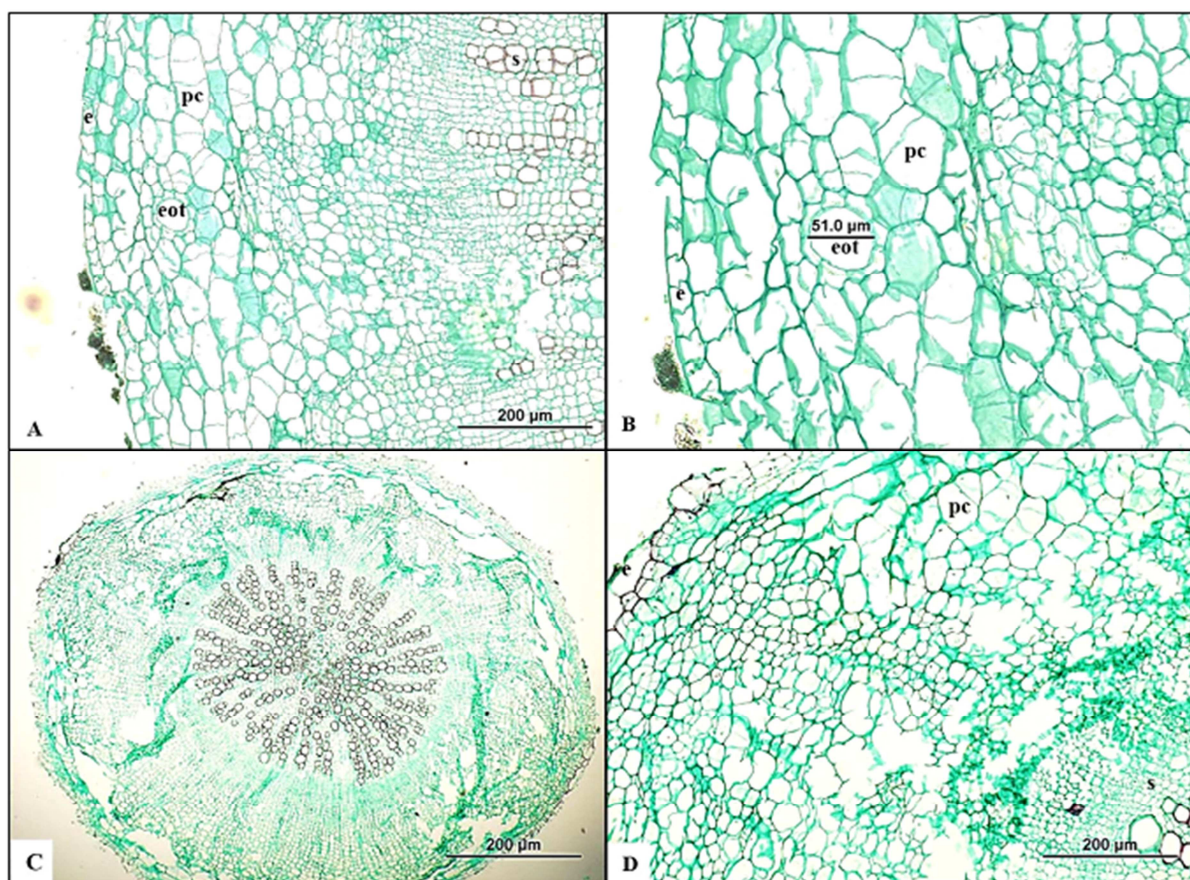


Fig. 3. Root transverse sections from Bay, Laguna (A-B) and La Trinidad, Benguet (C-D). e-epidermis; pc-parenchyma cells; s-stele; eot-essential oil-secreting tube cell.

The arrangement of *C. sativum* EOT cells being localized near the vascular system suggests that this pattern provide an efficient transport system. Some researchers suggest that internal secretory structures not within or in close proximity with the vascular bundles may have evolved mechanism to avoid diffusion limitations (Kromer et al., 2016; Lansing and Franceschi, 2000).

Comparison of Essential Oil-Secreting Tube (EOT)

Cells from Two Areas

Table 1 summarizes the number of EOT cells found in the vegetative organs of *C. sativum* from Bay, Laguna and La Trinidad, Benguet. In both areas, the stem has the most number of EOT cells, followed by the EOT cells in leaves, and the roots has the lowest EOT cells, in Bay. Localization of internal secretory structures, like essential oil-secreting tubes in association with vascular bundles was recognized in several species of Asteraceae (Andreucci et al., 2008; Cruz et al., 2014;

Kromer *et al.*, 2016). The EOT cells in stem were visible in both areas and, to a lesser extent in roots. In anatomical point, plant vascular tissues are systematically organized in continuous strands,

connecting from the root to the shoot system, and extending to every organ of the plant (Scarpella and Meijer, 2004).

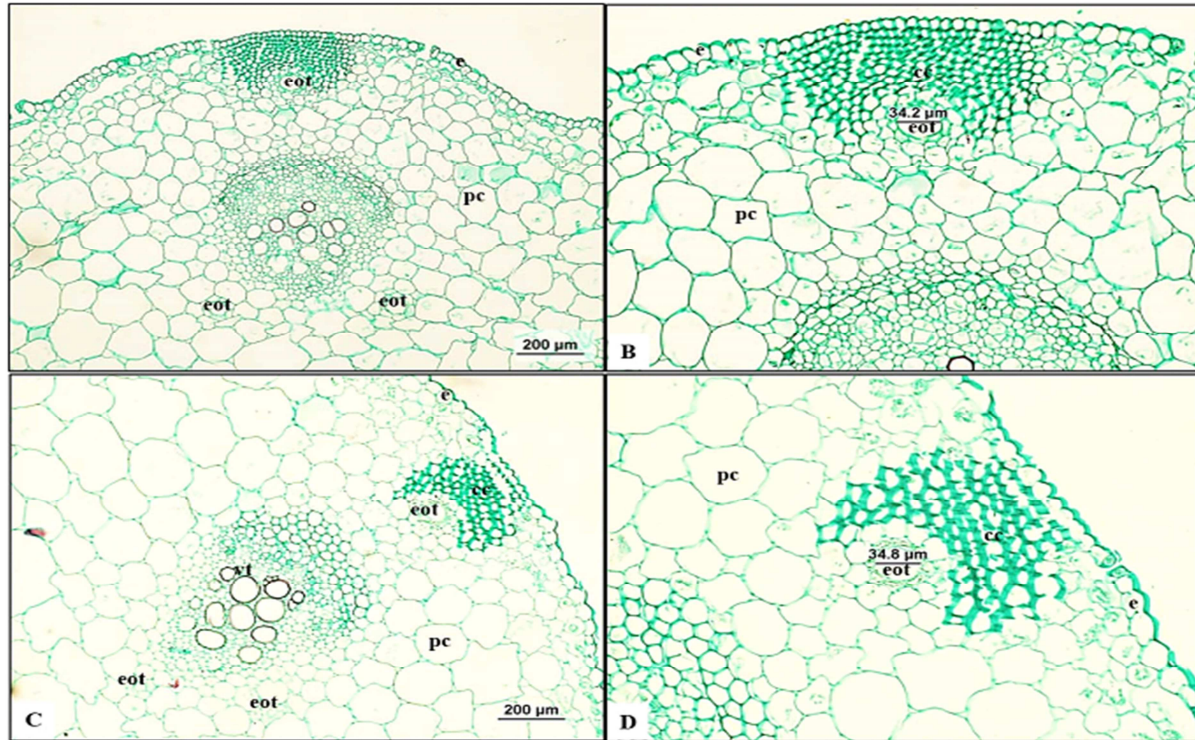


Fig. 4. Stem transverse sections from Bay, Laguna (A-B) and La Trinidad, Benguet (C-D). e-epidermis; pc-parenchyma cells; cc-collenchyma cells; vt-vascular tissue; eot-essential oil-secreting tube cell.

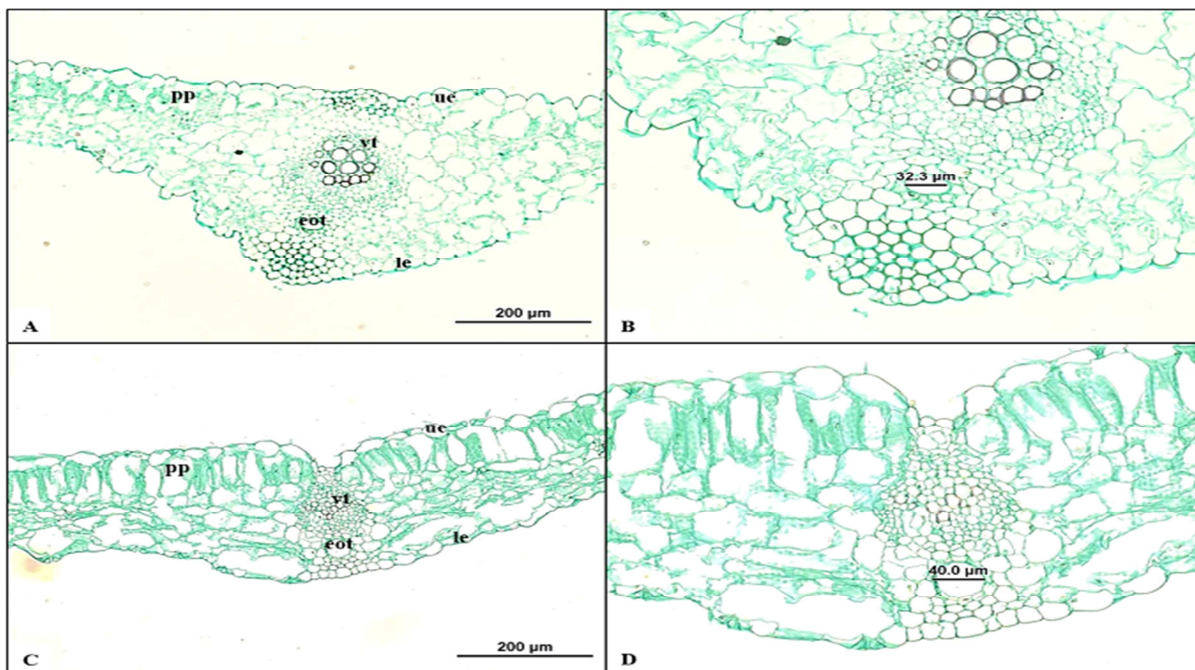


Fig. 5. Leaf transverse sections from Bay, Laguna (A-B) and La Trinidad, Benguet (C-D). upper epidermis; le-lower epidermis pp-palisade parenchyma; sp-spongy parenchyma-chlorenchyma cells; vt-vascular tissue; eot-essential oil-secreting tube.

The higher number of EOT cells examined from *C. sativum* stem can be explained by the divergence of vascular structures from the axial vascular cylinder toward the leaf veins, where EOT cells are observed to be localized along with the vascular bundles. Additionally, aside from the usual arrangement of EOT cells in proximity to vascular bundles, stem EOT cells can also be found below the collenchymatous cells of the hypodermis. The roots of *C. sativum* from Bay have the least number of EOT cells among the vegetative organs. In contrast, these cells were not

evident in the roots of *C. sativum* from La Trinidad. This can be attributed to the dilation of the parenchyma cells as it expands or might be due to the formation of aerenchyma. Little is known about the influence of altitude on the anatomy of internal secretory structures, especially those found in roots. Studies have shown increase in the diameter of root cortex and vascular tissues as an effect of wind in high altitude (GönüzandÖzörgücü, 1999). More studies are probably needed to establish the effect of altitude on internal secretory structures.

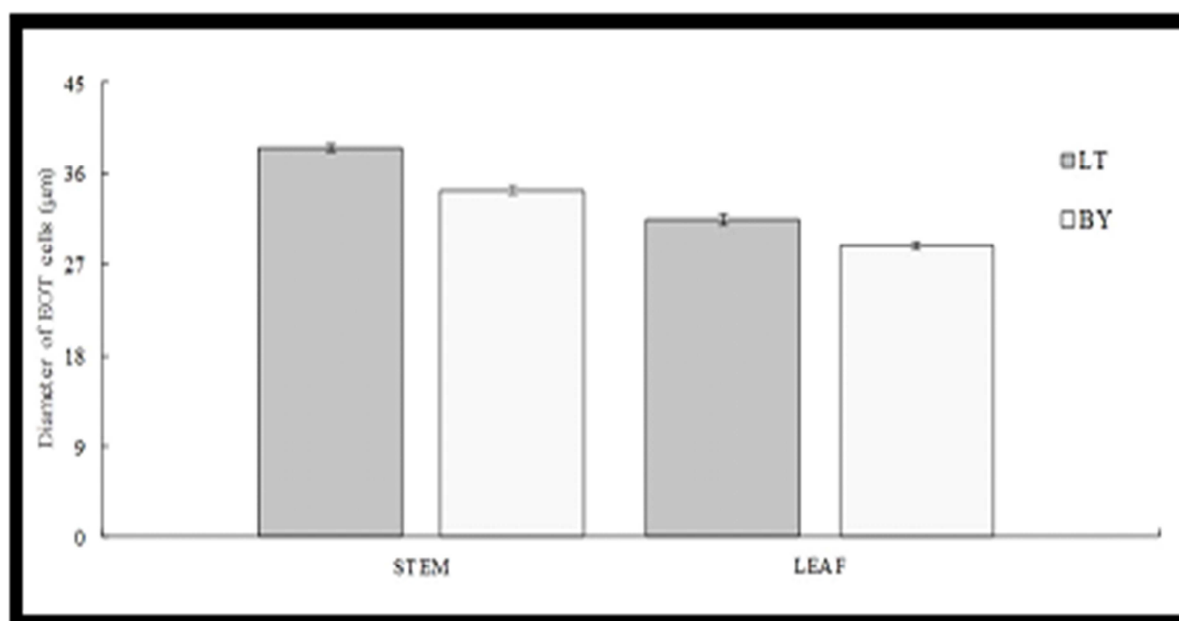


Fig. 6. Comparison on the diameter of stem and leaf EOT cells from Bay and La Trinidad. Values are means from three replications (n=45) and bars indicate standard errors.

Interspecies organ differences of EOT cells were observed in stem and leaves of *C. sativum*. EOT cells in stems are significantly larger ($p=0.001$) by 23% over the EOT cells in leaves. At 0.05 significance level, the mean diameter of stem EOT cells from La Trinidad (38.56 µm) are significantly larger compared to the EOT cells from Bay (34.33 µm). Similarly, EOT cells from leaves of *C. sativum* from Bay are significantly smaller (28.70 µm) than the EOT cells from La Trinidad, Benguet (31.31 µm) (Fig. 6). Morales-Payan (2010) reported that this herb grows faster and larger in temperatures between 20-25°C. This was also observed in our study where both diameter of essential oil-secreting tubes from stem and leaves sampled from La Trinidad, Benguet are

larger compared to those in Bay, Laguna. In *Tanacetumpolycephalum*, Mahdavi *et al.* (2013) reported that greater quantity of essential oil is extracted as the altitude increases. Moreover, biosynthesis of specific constituents of essential oils have been favored at high altitudes (Cruz *et al.*, 2014). Several studies have reported the varying responses of plant morphogenesis to altitudinal variations (Cruz *et al.*, 2014; Luo *et al.*, 2014). Specific environmental conditions during their ontogeny shapes phenotypic plasticity of plants, resulting to variation in anatomical structures. With increasing altitude, atmospheric pressure, temperature, photosynthetic active radiation (PAR), and humidity are observed to change and dominantly affect the anatomical

characteristics of plants. For instance, radiation and amount of precipitation increase with an increasing altitude together with the effects of wind, temperature differences, and humidity (Mahdavi *et al.*, 2013; Kromer *et al.*, 2016). In contrast, temperature and evaporation decrease with altitudinal increase. It is important to emphasize that, phenotypic plasticity in plants is influenced by several factors acting together. Thus, it is proposed that the temperature is possibly one of the factors that might influence the larger diameter of EOT cells in La Trinidad compared to samples from Bay.

Conclusion

The vegetative organs of *C. sativum* contain essential oil-secreting tube cells which produce the essential oil. Anatomically, the *C. sativum* roots, stems, and leaves sampled from two different altitude areas present the same tissue compositions, but with difference in the diameter of their essential oil-secreting tube cells. Essential-oil secreting tube cells are localized in the cortical region of the roots, below the hypodermal area of the stem and adjacent to its vascular bundles, and within the spongy parenchyma layer of the leaves. Essential oil-secreting tube cells in *C. sativum* roots are randomly arranged within the cortical region; ring-pattern in stems following the vascular bundle arrangement; and adjacent to vascular bundles in leaves. *C. sativum* stems and leaves from La Trinidad, Benguet which has higher elevation than Bay, Laguna have larger diameter essential oil-secreting tube cells compared to the latter.

These plant organs can also be utilized for producing essential oil particularly those regions which are having difficulties producing the reproductive organs due to temperature constraints. Further studies are vital to characterize the amount of essential oil produced from the different vegetative organs of *C. sativum*.

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