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# Comparative Anatomy of *Carpinus orientalis* Mill. (*Betulaceae*) Populations in Iran

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**Abstract**
Natural populations of *Carpinus orientalis* Mill. shrublands occur mainly in high and middle altitudes of the Hyrcanian forests, N. Iran, particularly on steep rocks and forest outcrops. There are some discrepancies on the intra-specific delimitation of this important woody species. The aim of the current study is to examine the anatomical variation of stems and leaves of sixteen populations of *Carpinus orientalis* collected in four north and northeastern provinces of Iran for the first time. It was shown that the anatomical characteristics of stem have some correlations with climatic variables such as temperature, altitude and precipitation, whereas midrib anatomical characteristics did not show any correlation with the above mentioned parameters.

**Key words:** Anatomical characters; *Carpinus orientalis;* Hyrcanian forest; Climatic factors

## Introduction

The genus *Carpinus* L. belongs to Betulaceae family (Yoo and Wen, 2002, Yoo and Wen, 2007; Chang, 2004); a well-defined family with six genera and 130 species (Chen, 1994). *Carpinus orientalis* Mill. is a small slow-growing deciduous tree or shrub that can be distinguished from its relative species, *C. betulus* L. due to having shorter and more base-branched trunks as well as growing mainly on open and rocky and drier outcrops (Sabeti, 1976; Browicz, 1982). This species distributes in southeastern Europe (Walters, 1964), Turkey on the Black Sea, Caucasus and north of Iran, from west to east of the Hyrcanian forest (Browicz, 1972).

Systematic application of anatomical features returns to about a century ago. One of the oldest information about systematic-anatomy is relevant to Solererder (1908) (Nejadhabibvash and Hosseini, 2009). Some anatomical features were already utilized for separation of taxonomical unites of a few woody species. Epidermal characters such as epidermal surface, structure of stomata and trichome frequency were utilized by Uzunova (1999) to separate *Corylus* L., *Carpinus* and *Ostrya* Scop. The anatomical features of *Salsola kali* subsp. *ruthenica* (*Chenopodiaceae*) proved that this species is well adapted to the harsh conditions (Bercu & Bavaru, 2004). Stem xylem features in two evergreen *Quercus* species and a deciduous one were analyzed along an Atlantic-Mediterranean climatic gradient by Villar-Salvador (1997). Rashidi *et al*. (2011) studied response of leaf anatomy in Ash to climatic factors. Ait Said *et al*. (2011) studied anatomical changes in *Pistacia atlantica* along an aridity gradient in Algeria. Metcalfe and Chalk (1957) described some common anatomical features of some genera of *Corylaceae* family. They mentioned that shape of petiole vascular strand varies in *Carpinus* and *Corylus*, cork arising in outer part of young stem cortex in certain species of *Corylus* and *Ostrya*. Secondary phloems stratified into alternating layers are consisting of strands of fibers and unthickened elements respectively in *Carpinus*, *Corylus* and *Ostrya*. However, the above-mentioned characteristics are very general and anatomy of *C. orientalis* has not been studied in details yet. This study, describe our effort to explore the anatomical responses of *Carpinus orientalis*

populations to a wide spectrum climatic factors including temperature, altitude and precipitation.
We collected samples from four large Iranian political provinces with distinct climates to demonstrate the relation between anatomical features of the populations with climatic factors.

## Materials and Methods

Samples of sixteen populations of *Carpinus orientalis* from four provinces of Northern Khorasan-e- Shomali, Golestan, Mazandaran and Gilan (from east to west of Iran) were collected in different habitats and altitudes during 2011-12 (Table 1; Fig. 1). Five trees were selected from each site and to prevent sampling from trees with same parents or pedigree, we adopted the approach suggested by Danquash (2011) and maintained a minimal distance of 100 m between each sampled tree. Leaves, petioles and young stems were fixed in Ethanol- Glycerin (1:1) for one month. Then cross sections were made in the middle region with a razor and stained with Methyl green and Congo red. After preparation of the slides, they were observed with Olympus light microscope, photographed by Dino-Eye camera model AM-423x and required characteristics were measured using Adobe Photoshop® ver.CS5. Table 2 represent the totl 53 anatomical characteristics that were measured through this study.
Climate data were extracted from Djamali *et al.* (2011). The climatic factors include temperature, precipitation and altitude. Ideas for selection of anatomical characteristics came from Khalili *et al*. (2010); Raei Niaki *et al*. (2009); Raoof ghotboddin *et al*. (2010) and Narimisa *et al*. (2009). Regarding quantitative and qualitative characters, five repeats of individuals of each population were measured and for all measurements, the average sizes were considered for quantitative and the most abundant for qualitative characters. Principal component analysis (PCA) results obtained from CANOCO software ver 4.5 were used to show populations on bi- dimensional graphs (Fig. 5). Statistical analyses were carried out using SPSS software (version 16).

## Results

### Lamina

Lamina mostly had a regular shape and arrangement. The following layers could be seen from up to down respectively: cuticle layer, upper epidermis, palisade parenchyma, spongy parenchyma, lower epidermis and cuticle layer.
*Epidermis*. The epidermis, which normally consist of a single layer of the cells forms outermost part of leaf. In the studied populations, epidermis is one-layered. The cells are variable in size and shape, which might be square or rectangular. The cells of upper surface are usually larger than those in the lower surface.
Cuticle thickness in the upper epidermis was more than that of lower epidermis in all of the studied populations.

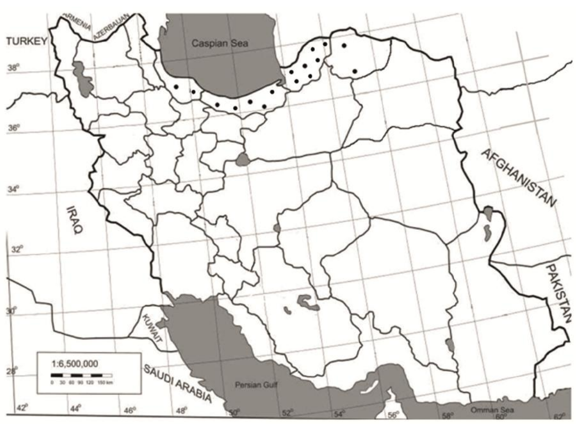


**Fig. 1.** Geographical location of studied specimens of *Carpinus orientalis* populations in Iran.

**Table 1.** Localities and features of each station of *Carpinus orientalis* population.

| Abbrev. | Province | Locality | Altitude (m a.s.l.) | Temperature | Precipitation (mm) |
|---|---|---|---|---|---|
| **A** | Golestan | National Park, Abshar, 55˚53'56"E, 37˚22'23"N | 700 | 17.8 | 573.8 |
| **B** | Khorasan-e shomali | Darkesh, Piazkuh, Gejji, 56˚43'34.23"E, 37˚25' 56.14"N | 1400 | 17.8 | 573.8 |
| **C** | Khorasan-e shomali | Darkesh, Kanigandi, 56˚43' 49.5"E, 37˚ 25' 45.5"N | 1300 | 17.8 | 573.8 |
| **D** | Mazandaran | Sari, Kiasar forest, 54˚48' 57"E, 36˚44' 35"N | 1000 | 16.2 | 1,359 |
| **E** | Mazandaran | Sari, Kiasar forest, 54˚ 48' 58"E, 36˚44' 36"N | 1400 | 17.8 | 601 |
| **F** | Gilan | Rasht, Rostamabad,Ammarlu, Sibon, Arbonav, Chichal, 56˚44' 53"E, 37˚25'10"N | 1600 | 18 | 702.6 |
| **G** | Gilan | Rasht, Rostamabad, Ammarlu, Sibon, Arbonav, Chichal, 56˚44' 54"E, 37˚25'11"N | 1800 | 17.8 | 601 |
| **H** | Golestan | National Park, Tangeh Gol, 55˚53' 56.81" E, 37˚22' 23.67" N | 600 | 16.2 | 1,359 |
| **I** | Golestan | Tangrah, near road, 55˚57'17.18" E, 37˚22' 8.62" N | 650 | 17.8 | 573.8 |
| **J** | Golestan | Gorgan, Qozloq river, 55˚53'56.81"E, 37˚22'23.67"N | 1100 | 18 | 702.6 |
| **K** | Golestan | Gorgan, Qozloq river, 55˚53'57.81"E, 37˚22' 24.67"N | 1300 | 17.8 | 601 |
| **L** | Golestan | Golestan: Gorgan, Zaringol valley, 54˚48'57.60" E, 36˚44'35.6"N | 904 | 17.8 | 601 |
| **M** | Golestan | Golestan: Gorgan, Mohammad Abad valley, 54˚48'57.60" E, 36˚44'35.6"N | 1050 | 16.4 | 1,293.50 |
| **N** | Mazandaran | Amol, Haraz road, Parand region, 56˚44' 28. 8"E, 37˚21'28. 7"N | 904 | 16.4 | 1,293.50 |
| **O** | Mazandaran | Amol, Haraz road, Parand region, 56˚44' 27.5"E, 37˚21'27.1"N | 805 | 17.8 | 601 |
| **P** | Mazandaran | Amol, Haraz road, Chelav region, 55˚57'17.18"E, 37˚22' 8.62"N | 840 | 18 | 702.6 |

**Table 2.** Descriptive data for quantitative characteristics used in the statistical analyses.

| Variable (mm) | Mean ±S.D. [Range] | Variable | Mean ±S.D. [Range] |
|---|---|---|---|
| Midrib upper epidermis width (MUEW) | 0.025± 0.034 [0.01-0.16] | Stem collenchyma thickness (SCT) | 0.15±0.90 [0.04-0.43] |
| Midrib upper epidermis length (MUEL) | 0.048± 0.03 [0.02-0.11] | Stem sclerenchyma fiber thickness (SSFT) | 0.061±0.025 [0.02-0.14] |
| MUEW / MUEL (REWL) | 0.546±0.380 [0.15-1.72] | Stem phloem diameter (SPHD) | 0.092±0.043 [0.03-0.19] |
| Midrib lower epidermis width (MLEW) | 0.033± 0.042 [0.01-0.18] | Stem xylem diameter (SXD) | 0.252±0.070 [0.11-0.36] |
| Midrib lower collenchyma thickness (MLCO) | 0.112± 0.039 [0.04-0.18] | Stem stele diameter (SSD) | 0.328±0.089 [0.13-0.46] |
| Midrib lower sclerenchyma thickness (MLSC) | 0.058±0.017 [0.04-0.10] | Stem diameter (SD) | 0.546±0.38 [0.15-1.72] |
| Midrib xylem's length (MX) | 0.135± 0.023 [0.10-0.19] | SSD / SD (RSD) | 0.227±0.062 [0.18-0.43] |
| Midrib phloem's length (MP) | 0.044± 0.009 [0.03-0.06] | Stem medula diameter (SBD) | 0.479±0.171 [0.14-0.79] |
| Lamina thickness (LT) | 0.281± 0.064 [0.16-0.39] | SBD / SD (RSBD) | 0.327±0.107 [0.23-0.7] |
| Palisade parenchyma length (PPL) | 0.102± 0.035 [0.03-0.16] | Petiole diameter (PD) | 0.937±0.463 [0.13-1.68] |

| | | | |
|---|---|---|---|
| Midrib length (ML) | 0.759±0.208 [0.75-1.02] | Stem collenchyma thickness (SCT) | 0.15±0.90 [0.04-0.43] |
| Midrib width (MW) | 0.609±0.162 [0.24-0.83] | Stem epidermis thickness (SSCT) | 0.1±0.101 [0.02-0.45] |
| Midrib upper cuticle's thickness (MUC) | 0.014±0.004 [0.01-0.02] | Spongy parenchyma length (SPL) | 0.114± 0.035 [0.6-0.21] |
| Midrib lower cuticle's thickness (MLC) | 0.009± 0.347 [0.01-0.16] | Stem cuticle thickness (SET) | 0.025± 0.012 [0.01-0.07] |
| Midrib lower epidermis width (MLEW) | 0.033± 0.042 [0.01-0.18] | Lamina lower cuticle's thickness (LC) | 0.003± 0.001 [0.00-0.00] |
| Midrib lower sclerenchyma thickness (MLSC) | 0.058±0.017 [0.04-0.10] | | |

*Mesophyll*. In the studied populations, mesophyll consists of one layer palisade parenchyma cells at the upper part and three layers of spongy parenchyma cells at the lower surface. Palisade cells were compact and elongate with straight walls, and spongy cells were polygonal or irregular and smaller in size (Fig. 2).

Palisade parenchyma length decreased in associated with higher precipitation, while spongy paranchyma length decreased. Spongy paranchyma length was decline with higher temperature.

## Midrib

Midrib mostly had a regular shape and arrangement. The following layers could be seen from up to down respectively: cuticle layer, upper epidermis, multi layer collenchymas, a layer parenchyma and sclerenchyma layer, xylem, phloem, pith, xylem, phloem, lower sclerenchyma, lower parenchyma, lower collenchymas, lower epidermis and cuticle layer.

*Vascular system*. This system is singular in Tangrah, Qozlogh (1100 m a.s.l), Qozlogh (1300 m a.s.l), Parand and Rasht (1800 m a.s.l) populations, while it posses two bundles in the remained populations. Shape of stele ranges from orbicular (Figs. 3b, 3h, 3i, 3l, 3m, 3o), semi-orbicular (Figs. 3a, 3f, 3p) to oblong, (Figs. 3c, 3d, 3e, 3g, 3j, 3k, 3n).

## Stem

Simple non-glandular trichomes cover the outer surface of epidermis of some populations. A transverse section taken from the young shoots showed occurrence of a single layer epidermis with thin cuticle in the outer surface, and layered collenchymas, one layer of parenchyma, a few sclerenchyma layers, phloem, xylem and layers of pith cells (Fig. 4).

*Stem diameter*. The stem diameter was measured by an average diameter of between 0.87 mm in Kiasar (1400 m a.s.l) to 2.22 mm in Chichal population (1600 m a.s.l).

Anatomical characters of stem showed the highest correlation to climatic factors (Fig. 5 & Table 3). Eigenvalues of first and second axes of PCA were 0.91 and 0.03 respectively. The two first axes explain 94.7 percent of variability of data. Increasing altitude associated with an increase in stem epidermis thickness and stem xylem diameter increased while stem diameter decreased. Stem phloem diameter and stem xylem diameter decreased with higher temperature. Also stem phloem diameter increased with higher rainfall while stem xylem diameter decreased (Fig. 5).

## Discussion

Up to now, there has been no comprehensive study about anatomical structure of *C. orientalis*. Among the researchers who studied this species, only Metcalfe and Chalk (1957) and Uzunova (1999) reported the general structure of Corylaceae and some *Carpinus* species. Anatomical structure of lamina revealed that the occurrence of single layer of the epidermis, a row of palisade parenchyma and three rows of spongy parenchyma was similar but number and shape of vascular bundles in the midribs. The structure of the lamina and midrib have not been mentioned in Metcalfe and Chalk (1957). The latter study only reported that epidermis mucilaginous in species of *Carpinus* and *Corylus* and vascular bundle of the large vein accompanied by arcs of sclerenchyma. Furthermore, according to our results lamina and stem anatomical features are in compatible with the climatic parameters (Fig. 6, 7, 8). The result is in consistent with studies of Rashidi *et al* (2011). Plants living in regions characterized with low rainfall have more palisade parenchyma than spongy parenchyma (Rashidi *et al*, 2011). However, anatomical features respond differently to climatic gradient. For example, Villar-Salvador *et al*. (1997) showed that the responses of *Quercus ilex* and *Q. coccifera* stem xylem were different along a certain climatic gradient.

**Table 3.** Pearson correlation matrix between environmental variables and anatomical features of *Carpinus orientalis:* ML- Midrib length; MW- Midrib width; MUC- Midrib upper cuticle's thickness; MLC- Midrib lower cuticle's thickness; MUEW- Midrib upper epidermis width; MUEL- Midrib upper epidermis length; MLEW- Midrib lower epidermis width; MLCO- Midrib lower collenchyma thickness; MLSC- Midrib lower sclerenchyma thickness; MP- Midrib phloem's length; LT- Lamina thickness; LC- Lamina lower cuticle's thickness; PPL; Palisade parenchyma length; SPL- Spongy parenchyma length; SET- Stem cuticle thickness; SCT- Stem collenchyma thickness; SSCT- Stem epidermis thickness; SSFT- Stem sclerenchymal fiber thickness; SPHD- Stem phloem diameter; SXD- Stem xylem diameter; SSD- Stem stele diameter; SD- Stem diameter; RSD- SSD / SD; RSBD- SBD / SD; RSPH- SPHL / SSD; PD- Petiole diameter.

| | **Altitude** | **Precipitation** | **Temperature** | **Continentally index** |
|---|---|---|---|---|
| ML | | | -.296* | |
| MW | | | -.257* | |
| MUC | -.284* | | .287* | -.311* |
| MLC | -.314* | | 0.202 | -0.161 |
| MUEW | | -.424** | -.356** | .311* |
| MUEL | .262* | -0.193 | -0.228 | 0.155 |
| REWL | | -.327** | -.258* | .327** |
| MLEW | | -0.029 | 0.07 | |
| MLCO | | -.315* | -.248* | |
| MLSC | | -.516** | -.357** | |
| MP | .277* | -.261* | -.248* | |
| LT | .290* | | -.367** | |
| LC | | | -.253* | |
| PPL | | .396** | | |
| SPL | | -.256* | -.292* | .387** |
| SET | | | -.276* | |
| SCT | | | -0.06 | |
| SSCT | .358** | | -.313* | |
| SSFT | | | -0.06 | |
| SPHD | | -.482** | -.370** | .476** |
| SXD | .354** | -.338** | -.495** | .272* |
| SSD | | -.402** | -.555** | .293* |
| SD | -.384** | | | |
| RSD | .421** | -.582** | -.646** | |
| SBD | | -.366** | -.461** | |
| RSBD | .370** | -.572** | -.622** | |
| RSPH | | -.272* | | .354** |
| PD | .566** | -.270* | -.435** | .291* |

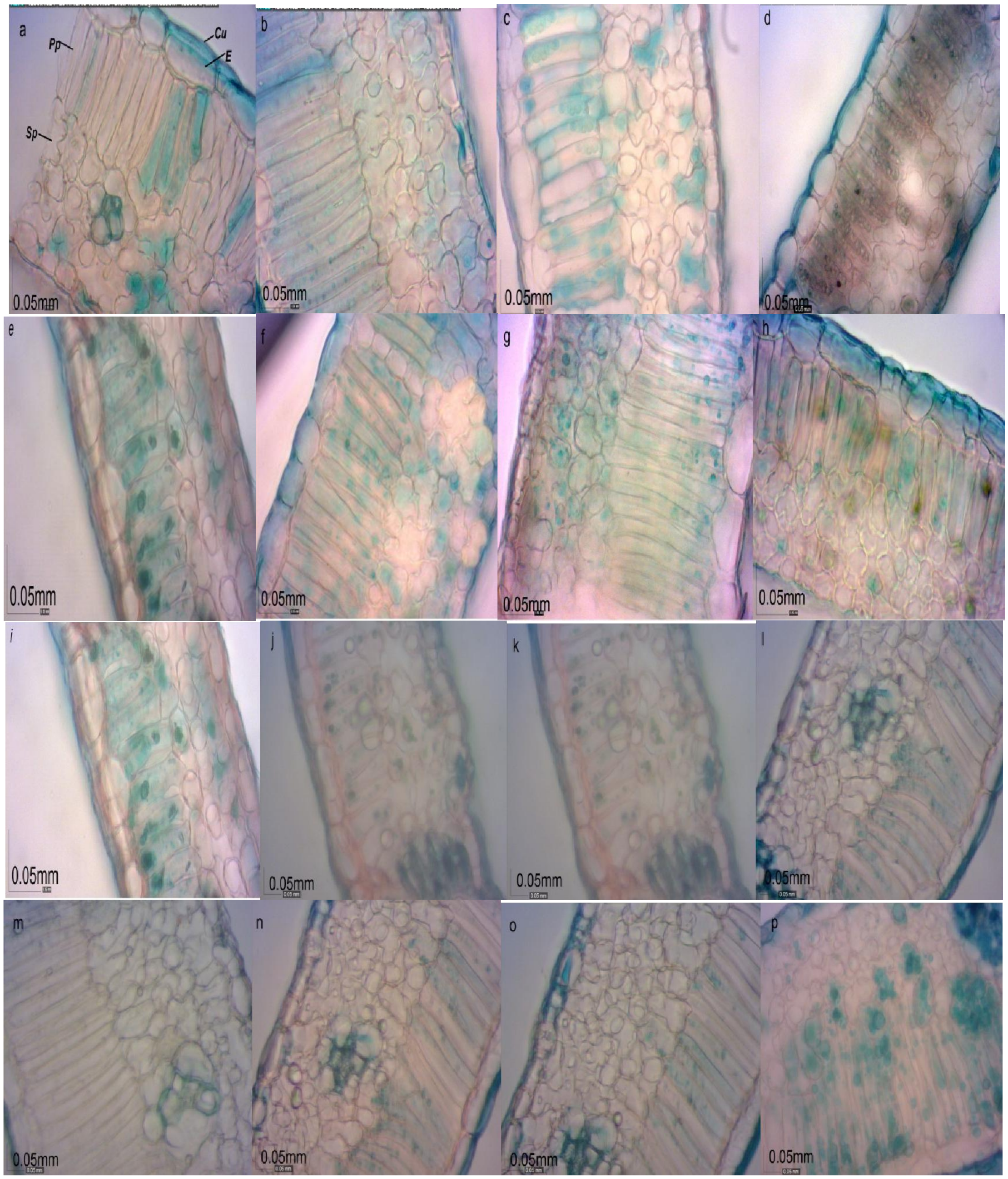


**Fig. 2.** Lamina cross sections in the populations of *C. orientalis*: a, Abshar; b, Gheji; c, Kanigandi; d, Kiasar (1000 m a.s.l); e,Kiasar (1400 m a.s.l); f, Rasht (1600m a.s.l); g, Rasht (1800 m a.s.l); h, Tangeh Gol; I, Tangrah; j, Qozlogh (1100 m a.s.l); k, Qozlogh (1300 m a.s.l); l, Zaringol; m, Mohammad Abad; n, Parand (904 m a.s.l); O, Parand (805 m a.s.l); P, Chelav; Cu – cuticle; E – epidermis; Pp – Palisade parenchyma; Sp – Spongy parenchyma.

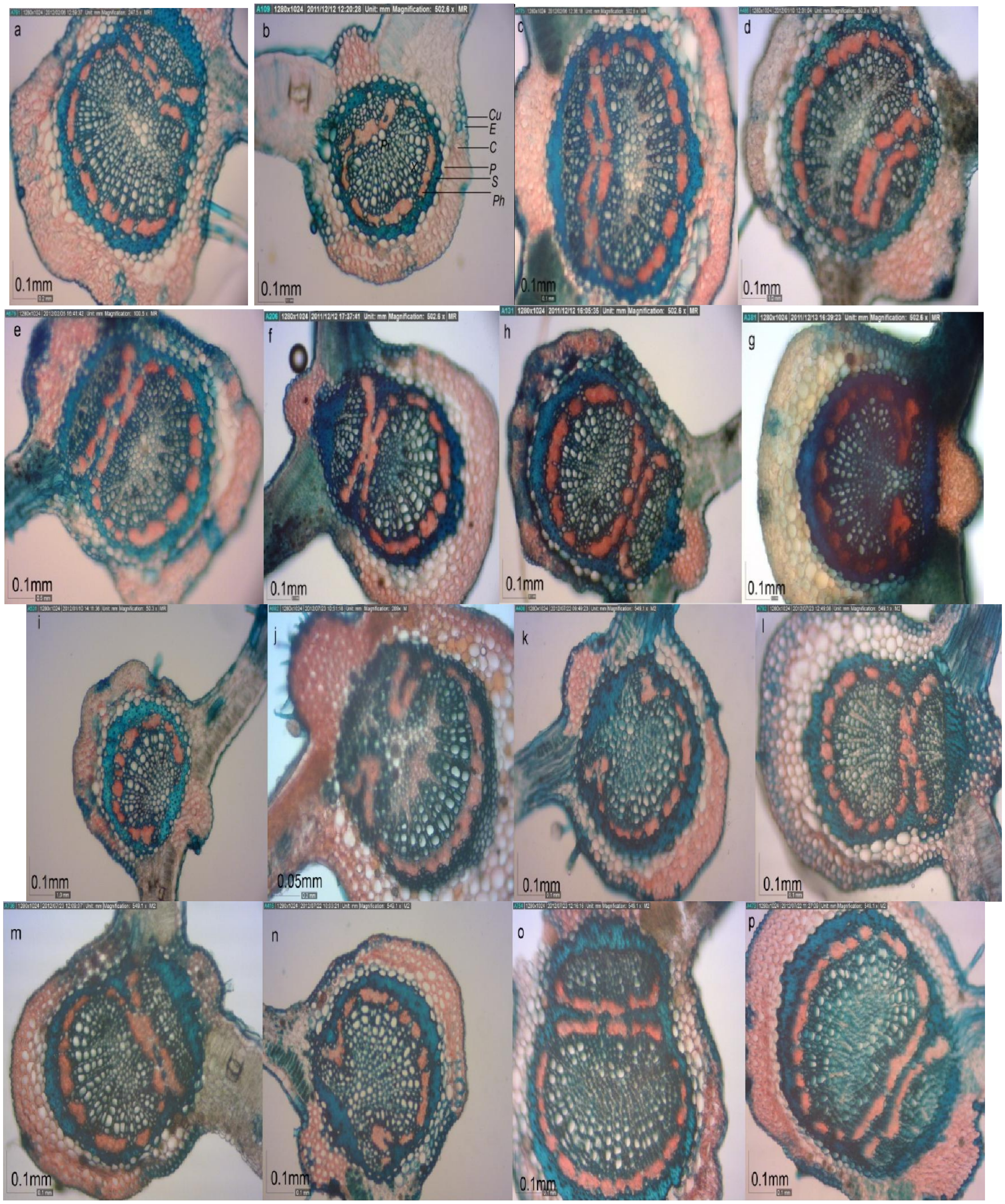


**Fig. 3.** midrib cross sections (midribs) in the populations of *C. orientalis*: a, Abshar; b, Gheji; c, Kanigandi; d, Kiasar (1000 m a.s.l); e,Kiasar (1400 m a.s.l); f, Rasht (1600 m a.s.l); g, Rasht (1800 m a.s.l); h, Tangeh Gol; I, Tangrah; j, Qozlogh (1100 m a.s.l); k, Qozlogh (1300 m a.s.l); l, Zaringol; m, Mohammad Abad; n, Parand (904 m a.s.l); O, Parand (805 m a.s.l); P, Chelav. Cu – cuticle; E – epidermis; P – parenchyma; C – collenchymas; S – sclerenchyma; PH – phloem; X- xylem.

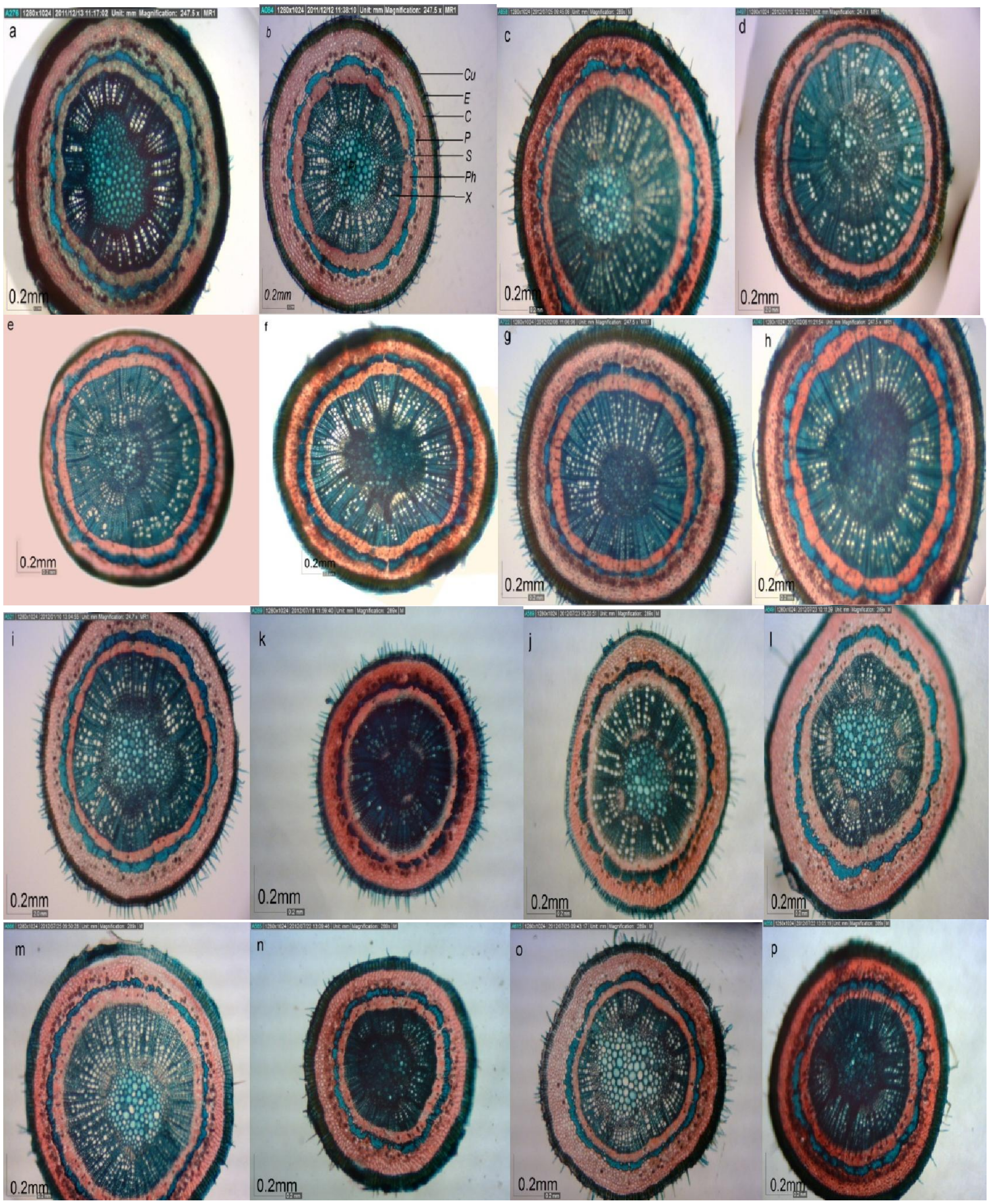


**Fig. 4.** Stem cross sections in the populations of *C. orientalis*: a, Abshar; b, Gheji; c, Kanigandi; d, Kiasar (1000m a.s.l); e,Kiasar (1400m a.s.l); f, Rasht (1600m a.s.l); g, Rasht (1800m a.s.l); h, Tangeh Gol; I, Tangrah; j, Qozlogh (1100m a.s.l); k, Qozlogh (1300m a.s.l); l, Zaringol; m, Mohammad Abad; n, Parand (904 m a.s.l); O, Parand (805m a.s.l); P, Chelav, Cu – cuticle; E – epidermis; P – parenchyma; C- collenchymas; S – sclerenchyma; PH – phloem; X- xylem; Pi – pith.

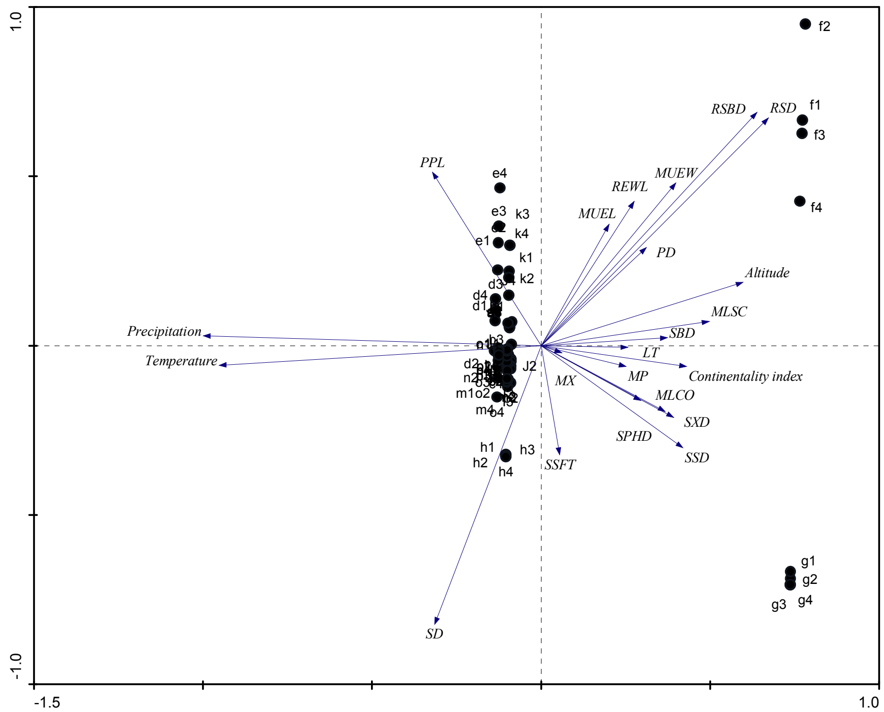


**Fig. 5.** PCA analysis of 16 populations of *Carpinus orientalis*: **MUEW**- Midrib upper epidermis width; **MUEL-** Midrib upper epidermis length; **MLEW**- Midrib lower epidermis width; **MLCO-** Midrib lower collenchyma thickness; **MLSC-** Midrib lower sclerenchyma thickness; **MP-** Midrib phloem's length; **LT**- Lamina thickness; **PPL**; Palisade parenchyma length; **SSCT**- Stem epidermis thickness; **SSFT**- Stem sclerenchymal fiber thickness; **SPHD-** Stem phloem diameter; **SXD-** Stem xylem diameter; **SSD-** Stem stele diameter; **SD-** Stem diameter; **RSD**- SSD / SD; **RSBD**- SBD / SD; **RSPH**- SPHL / SSD; **PD**- Petiole diameter

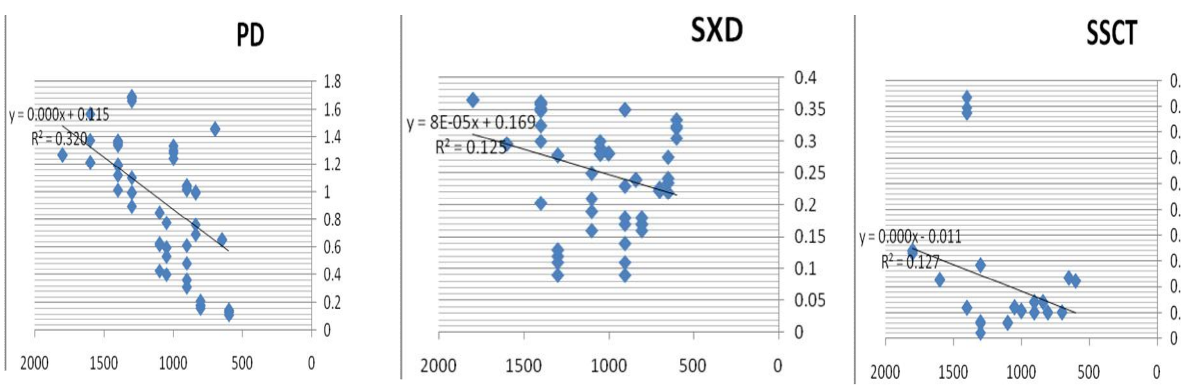


**Fig. 6**. Relationship between SXD, PD, SSCT and altitude in *C. orientalis*: SXD- Stem xylem diameter; PD- Petiole diameter; SSCT- Stem epidermis thickness


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