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Anatomical Studies on Three Jerusalem Artichoke (*Helianthus tuberosus* L.) Cultivars Grown in Hungary

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JERUSALEM Artichoke, a member of the family Asteraceae, is classified as a foodstuff (tubers), animal feed (fodder or silage), and an energy crop for bioethanol production. This crop has several economic and medicinal benefits including the production of inulin, fructose, and proteins. It could be utilized as raw materials for the chemical, pharmaceutical and food industries. The anatomical differences between the three culivars (Alba, Balady and Fuza) were investigated. Balady and Fuza cvs showed the highest main values of the most stem anatomical parameters as well as conductive tissues thickness (xylem and phloem). The highest main values of mesophyll tissue thickness (palisade and spongy tissues) and stomatal distribution were noted in Balady and Alba. The obtained results showed that, Balady cultivar has superiority in the most of stem and leaf anatomical parameters, which may make the Balady cultivar of *H. tuberosus* more tolerant to abiotic stress conditions.

Keywords: Jerusalem artichoke, Conductive tissues, Mesophyll tissue, Stomat

1. Introduction

Jerusalem artichoke (JA) is a non-grain perennial plant belonging to the family Asteraceae (Abdalla et al. 2014; Qiu et al. 2018). The plant is a crucial source for human and animal nutrition (Kaszás et al. 2018; Abdalla et al. 2020), and an important feedstock for biorefinery (Johansson et al. 2015; Qiu et al. 2018; Rossini et al. 2019; Wang et al. 2020), Also it is an effective ecological restoration crop (Shao et al. 2021). Moreover, it is an excellent candidate crop due to its high adaptability to rapid growth, high commercial value, and its low requirements of water and fertilizers (Zhao et al. 2020). Additionally, it is characterized with high photosynthetic efficiency, high yield (Shao et al. 2021), and resistance to abiotic stresses including drought (Zhao et al. 2021), salinity (Shao et al. 2019, 2021), and low temperatures (Mu et al. 2021).

Recently, El-Ramady et al. (2021) highlighted the importance of the anatomical features and their association with the morphology and physiology of plants grown under different environmental conditions. Although the discipline of plant anatomy or phytotomy is an important biological plant science that improves our understanding of plant growth under complex environmental conditions (Lisztes-Szabó 2019).

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Moreover, it provides a better understanding of the interrelationships of plants with the surrounding environments (Bákonyi et al. 2020; El-Ramady et al. 2021). Recently, several studies have focused on the plant anatomy under different conditions or stresses such as bio-organic fertilization (Bákonyi et al. 2020), Zn-stress (dos Santos et al. 2020), water deficit (Bueno et al. 2020; Lobato et al. 2020), and salinity stress (Moura et al. 2021), as well as study the differences between crop cultivars (Bhusal et al. 2020; Rouinsard et al. 2021).

This study is an attempt for histological characterization of three cultivars of JA using some different anatomical measurments of stem, leaves and stomata. This study also emphasizes whether these anatomical features are considered strong indicators for differentiation between the different JA cultivars grown under the same environmental conditions.

2. Materials and Methods

2.1 Plant materials

Three cultivars of Jerusalem artichoke (i.e., Alba. Balady, and Fuza) were examined in this study. Tubers of Balady and Fuza were obtained from Agricultural Research Center in Giza and local farm in Ismailia, Egypt, respectively, while Alba was obtained from Debrecen University, Hungary. The tubers of these cultivars were cultivated in the Demonstration Garden, Agricultural Botany, Plant Physiology and Biotechnology Department, Debrecen University, Hungary at the end of April 2014. Because JA is a perennial crop, the tubers were stored in the soil during the winter seasons. In spring (the end of April) of the next year 2015, the stored *in situ* sprouted tubers in the soil were re-cultivated for five years in the same place in the garden. The plants were irrigated using the natural rainwater and no fertilizers were applied during this period.

2.2 Preparation of leaf and stem samples for anatomical investigation

The shoots from each JA cultivar at the vegetative stage (Abdalla et al. 2020) were randomly collected at the end of June 2019. Stem segments were taken from the second internode from shoot tips, whereas leaf samples were taken from two different positions on the shoot starting from the shoot tip (top and bottom) for each cultivar too. Stem and leaf samples were kept in preservation solution (water: glycerine: ethanol (1:1:1; v/v/v) in a glass bottle for one month (Fig. 1, photo 2). Each glass bottle contained three stem segments or three leaves which represent three replicates for each cultivar.

2. 3 Preparation of stem and leaf slides for histological examination

Two drops of distilled water were put on the stem segment which was placed on the glass slide. The stem segment was cut horizontally using sharp blade into very thin transections that were put at once in distilled water until finish cutting. After that, the very thin transections of stem were selected and were soaked in hypo solution (sodium hypochlorite: distilled water at volume ratio 1:1) for one hour (Fig. 1, photo 4) for removing chlorophyll and other pigments if exist or until the sample could be very clear or transparent, where natural pigments must be removed from the transections to be able to accept dyeing and colored well with the required pigments. Then, transections were washed gently four times using distilled water and kept until to be suitable for staining. While for preparing leave slides, we have already followed the next protocol which little different from that used for the stem. Because it was noticed those tissues of preserved JA leaves were very soft and there weren't easy to cut for making slides. For this reason, leaves were removed from the preservation solution then they were washed using distilled water. After that, they were dipped in ethanol alcohol 70% for overnight to be hard enough for cutting (Fig. 1, photo 3). After that, they were washed well with distilled water. The leaf was placed on a clean slide and cut manually using a new and sharp blade after putting a drop of distilled water on it. Where, the two sides of the leaf were cut leaving just one cm including the mid-vein of the leaf. This part of the leaf had been cut into thin transections. These transverse sections were soaked in hypo solution (sodium hypochlorite: distilled water at volume ratio 1:1, Fig. 1, photo 4) for 30 min because they were very sensitive to hypo solution as it causes its tearing and decaying. Careful washing must be done to leave transections four times with distilled water to be ready for staining.

2.4 Staining of samples for microscopic observations

Selected very thin stem or leaf transections were dipped in one drop of toluidine blue 0.2% and three drops of distilled water for a few

seconds (Fig. 1, photos 5 and 6). Well stained transverse sections were selected and 3-4 transections were placed on a clean slide. One drop of distilled water was added after that, clean glass cover was put over transections. For anatomy measurements, stem and leaf slides were examined efficiently using a light microscope (Zeiss Axioscope 2+; Zeiss International, Oberkochen, Ostalbkreis, Germany) with a compatible camera, and the Scope Photo software (Scopetek, München, Germany) was used for photographing the images. Stem diameter, cortex tissue thickness, xylem tissue thickness, collenchyma tissue thickness, phloem tissue thickness, xylem vessels diameter were measured for stem transections (all parameters in µm). Leaf lamina thickness, middle midrib xylem tissue thickness, middle midrib phloem tissue thickness, palisade tissue thickness, spongy tissue thickness were measured for leaf transections (all parameters in µm).

2.5 Preparation of stomata slides

Likewise, some random shoots from each cultivar of JA plant at the vegetative stage; were collected at the beginning of September 2019 (Fig. 1, photo 1), fresh leaf samples were separated for preparing stomata slides. Stomata slides were made by selecting the suitable leaves on the shoots on the sixth and the seventh internode from the shoot tip. By using a thin layer of transparent nail polish, the epidermal layer could be separated after drying up the polish. Number of stomata/250 µm^2 of upper epidermis and epidermis of JA leaves of the three studied cultivars were calculated.

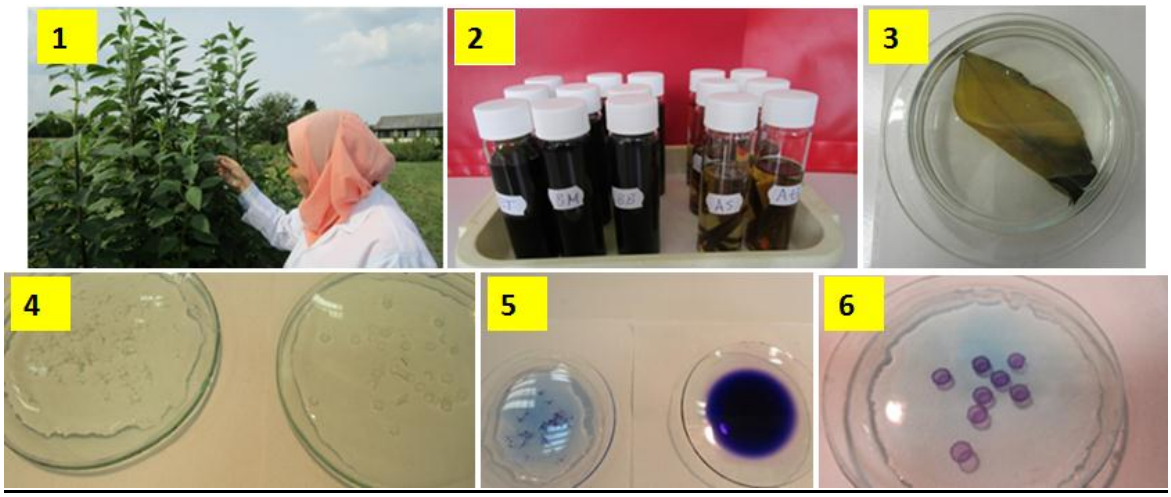

Fig. 1: Photo (1): JA plant at vegetative stage as a source of fresh leaf samples, September 2019 for preparing stomata slides. Demonstration Garden, Agricultural Botany, Plant Physiology and Biotechnology Department, Debrecen University, Hungary. Photo (2): Samples of stem and leaves of JA cultivars in preservation solution. Photo (3): JA leaf kept in ethanol 70% for overnight. Photo (4): leaves and stem transections soaked in hypo solution. Photo (5): Selected very thin leaf transections dipped in one drop of toluidine blue 0, 2% + three drops of distilled water for few seconds for staining. Photo (6): Well stained stem transections in distilled water ready for microscopic examination.

3. Results and Discussion

3.1 Stem anatomical studies

The internal structure of the stem and leaf blade of JA is similar to other dicotyledonous plants. Data illustrated in Fig. 2 and presented in Table 1 show the anatomical differences between three cultivars of JA (i.e., Alba, Balady and Fuza). The highest mean values of cross section diameter, hypodermal collenchyma, conductive tissues thickness and xylem vessels diameter were obtained in Balady and Fuza cultivars, while the lowest mean values were indicated in Alba cultivar. The highest mean values of cortex and xylem tissues thickness were recorded in Alba cultivar. The wider xylem vessels

diameter was found in Balady and Fuza cultivars. Non-glandular multicellular unbranched trichomes were shown on the stem of Balady cultivar.

The observed increment in stem diameter is due to the increased thickness of cortex, vascular (phloem and xylem) tissue resulted from stimulation of cambial cell activity. Collenchyma is the main supporting tissue of growing organs with walls thickening during and after elongation and highly dynamic, especially compared with sclerenchyma (Leroux 2012). Collenchyma tissue generally consists of living cells, while sclerified tissues generally consist of dead cells with non-extensible rigid cell walls that are unable to undergo mitotic divisions. In small slowly growing plant organs such as dicotyledonous stems and branches, turgor pressure generated in parenchyma cells may provide sufficient support, but many plant stems grow fast and are fragile, and therefore they cannot fully rely on turgor pressure for support (Jarvis 2007). Fahn (1990) mentioned that chloroplasts are present in collenchyma, but in typical collenchyma tissue with a clear mechanical function, chloroplasts are rarely found (Evert 2006). However, to allow photosynthesis, collenchyma cell walls are generally translucent, enabling light to be transmitted to the chloroplasts in the tissues below (Leroux 2012).

The highest thickness values of xylem and phloem were recorded in Balady and Fuza cultivars. Xylem and phloem tissues are vascular or conductive tissue formed from embryonic tissues, called procambium and vascular cambium. The procambium is the apical meristem that produces the primary phloem and primary xylem in the embryo and in the young portions of shoots and roots. The vascular cambium is a lateral meristem found in gymnosperms and dicotyledons, where it produces the secondary phloem and secondary xylem.

The diameter of xylem vessels is an important parameter for evaluating the ascent of sap and the adaptation of plants to their environment. The cambial division leads to increased production of xylem biomass (Milhinhos and Miguel 2013). Xylem and phloem tissues differentiation can be controlled by interactions of phytohormones, such as auxins, gibberellins, cytokinins, and ethylene (Patel et al. 2014).

Environmental factors have an impact on the initiation and differentiation of vascular cambium. The rate of cambial cell division and, in turn, xylem development, is correlated with temperature and drought. Cambial activity of woody plants is very sensitive to water deficits and drought decreases or delays cell division of vascular cambium by reducing turgor pressure of cambial cells, leading to reduced plant growth (Patel et al. 2014). Since it is composed of non-living cells that cannot acclimate to the changing environmental factors, conditions at the timing of xylogenesis are important (Mirwais et al. 2019).

Table 1: Some stem anatomical parameters of three Jerusalem artichoke cultivars (Alba, Balady and Fuza)

Cultivars	Stem cross section diameter (µm)	Cortex tissue thickness (µm)	Xylem tissue thickness (µm)	Collenchyma tissue thickness (µm)	Phloem tissue thickness (µm)	Xylem vessels diameter (µm)
Alba	339.74±0.04	37.27±0.02	10.106±0.003	16.541±0.003	9.645±.002	3.389±0.001
Balady	347.51±0.01	38.74±0.01	10.246±0.002	18.868±0.001	10.702±0.007	4.382±0.002
Fuza	352.65±0.01	38.81±0.01	15.084±0.004	22.209±0.001	15.042±0.002	4.385±0.001
F test	**	**	**	**	**	**
LSD $_{0.05}$	0.039	0.034	0.008	0.003	0.006	0.003

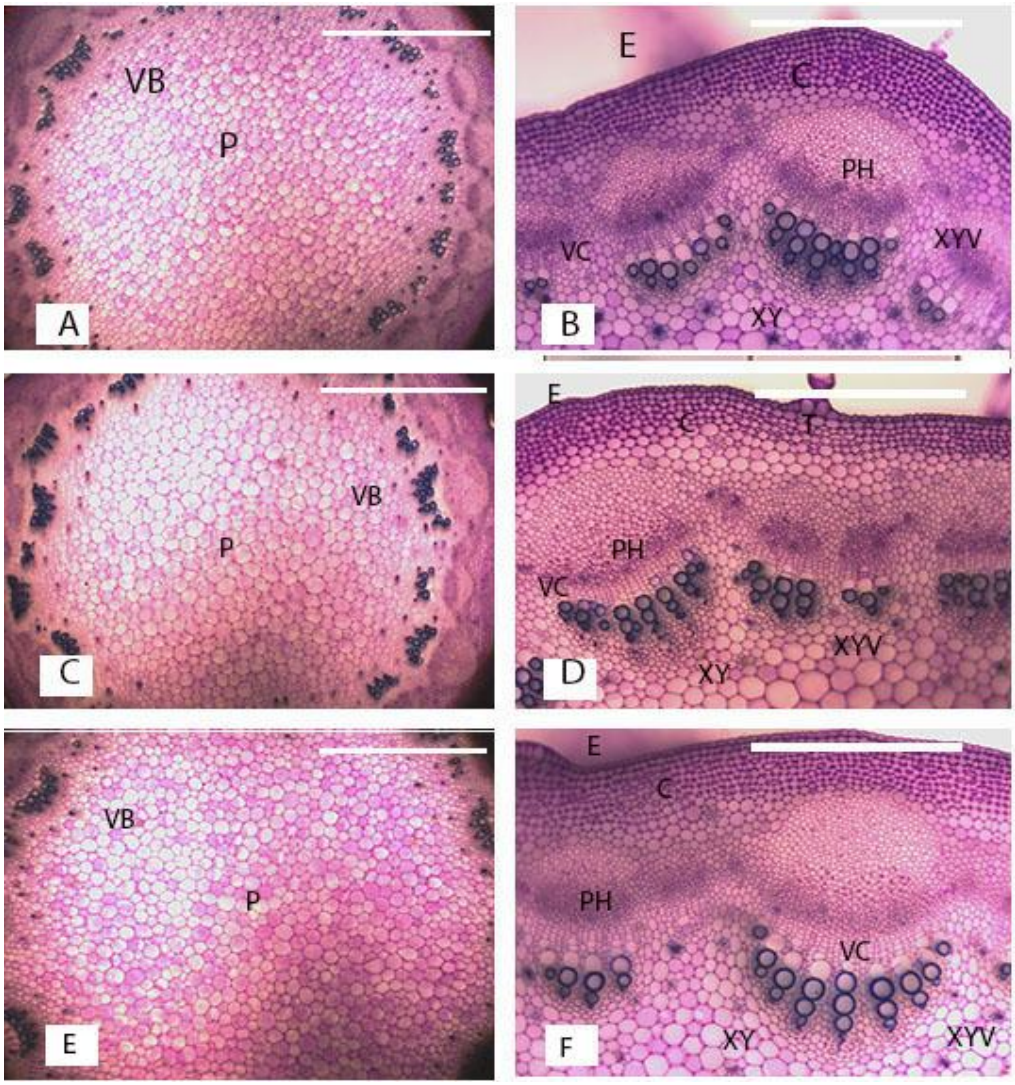

Fig. 2: Photomicrographs of stem cross section of three Jerusalem artichoke cultivars, A and B: Alba, C and D: Balady, E and F: Fuza, c: collenchyma tissue, E: epidermis, PH: Phloem tissue, T: Trichome, VC: vascular cambium, XY: xylem tissue, XYV: xylem vessels (Bars: A, C and E=100 µm; B, D and F=50 µm). The stem cross sections have been prepared, stained by toluidine blue (0, 2%), examined using light microscope (Zeiss Axioscope 2+; Zeiss International, Oberkochen, Ostalbkreis, Germany) and photographed using Scope-Photo software ((Scopetek, München, Germany) by Neama Abdalla.

3.2 Leaf anatomical studies

The analyses of the internal leaf anatomical structure of the three different JA cultivars are presented in Fig. 3 and Table 2. Thicker leaf lamina, palisade and spongy tissues and vascular tissues (middle midrib vascular bundle) were shown in Balady and Alba cultivars. The highest thickness values of mesophyll tissue (palisade and spongy tissue) were recorded by Balady cultivar. The midrib cross section in Balady seems to be wider than in the other two cultivars (Fig. 3C). Leaf lamina of JA had palisade and spongy tissues (mesophyll tissue). The mesophyll generally is differentiated into irregularly shaped spongy parenchyma cells and columnar palisade parenchyma cells. The spongy parenchyma tissue usually is located on the upper and the lower epidermis, and the palisade parenchyma on the below the upper epidermis tissue. The main task of mesophyll tissue (chlorenchyma tissue) is the photosynthesis process (Crang et al. 2018). The differences between Alba, Balady and Fuza

cultivars might include increasing in internal photosynthetic surface area leaf lamina thickness and number of chloroplastide/cell as well as chlorophyll pigment contents (Yan et al. 2012).

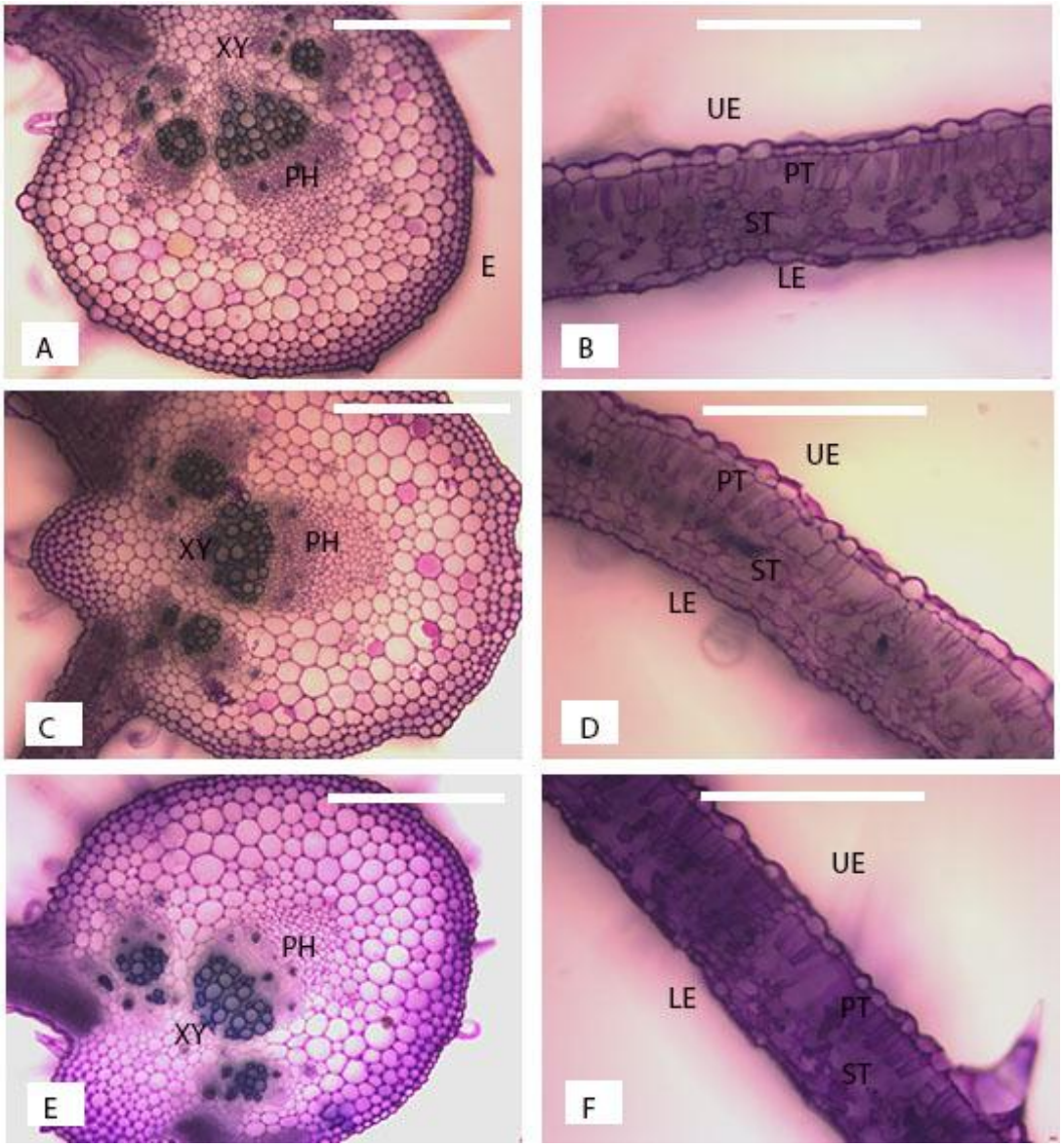

Fig. 3: Photomicrographs of leaf lamina cross section of three Jerusalem artichoke cultivars, A and B: Alba, C and D: Balady, E and F: Fuza, UE: upper epidermis, LE: lower epidermis PH: Phloem tissue, XY: xylem tissue, PT: palisade tissue, ST: spongy tissue (Bars: A, C and E=100 µm; B, D and F=50 µm). The leaf cross sections have been prepared, stained by toluidine blue (0, 2%), examined using light microscope (Zeiss Axioscope 2+; Zeiss International, Oberkochen, Ostalbkreis, Germany) and photographed using Scope-Photo software ((Scopetek, München, Germany) by Neama Abdalla.

Table 2: Some leaf anatomical parameters of three Jerusalem artichoke cultivars.

Cultivars	Leaf lamina thickness (µm)	Middle midrib xylem tissue thickness (µm)	Middle midrib phloem tissue thickness (µm)	Palisade tissue thickness (µm)	Spongy tissue thickness (µm)
Alba	58.742±0.003	46.447±0.002	32.958±0.002	18.954±0.004	31.305±0.003
Balady	64.655±0.003	42.302±0.002	32.871±0.01	22.140±0.002	34.545±0.004
Fuza	55.272±0.002	42.216±0.002	24.297±0.002	13.875±0.004	30.707±0.002
F test	**	**	**	**	**
$LSD_{0.05}$	0.006	0.005	0.005	0.031	0.008

3.3 Stomata distribution

As adjustable pores, each delimited by a pair of guard cells, stomata are located usually in high frequency on lower epidermis tissue. Stomata distribution on lower and upper leaf surface among the three different JA cultivars were determined. Data in Fig. 4 and Table 2 indicated that stomata occur on both leaf surface sides (amphistomatous leaves). Stomatal frequency is greater in the lower leaf surface than the upper leaf surface. The highest stomatal density values were recorded on both leaf surface sides by Alba and Balady. Stomata (singular stoma), are central determinants of plant photosynthesis, transpiration and ecological adaptability, CO_2 uptake during photosynthesis, and thereby water relation and plant biomass accumulation is influenced by stomatal movement (Al Afas et al. 2006; Bussis et al. 2006). An advantage of distributing stomata over both leaf surfaces is to double the boundary layer conductance; hence, amphistomatous leaves could be advantageous in environments when photosynthetic rates can be potentially limited by a low leaf conductance, as could be the case in leaves exposed to high irradiance (Camargo and Marenco 2011). These anatomical differences among the three tested JA cultivars could mainly be attributed to both the differences in their genetical constitution and their response to the surrounded environmental conditions.

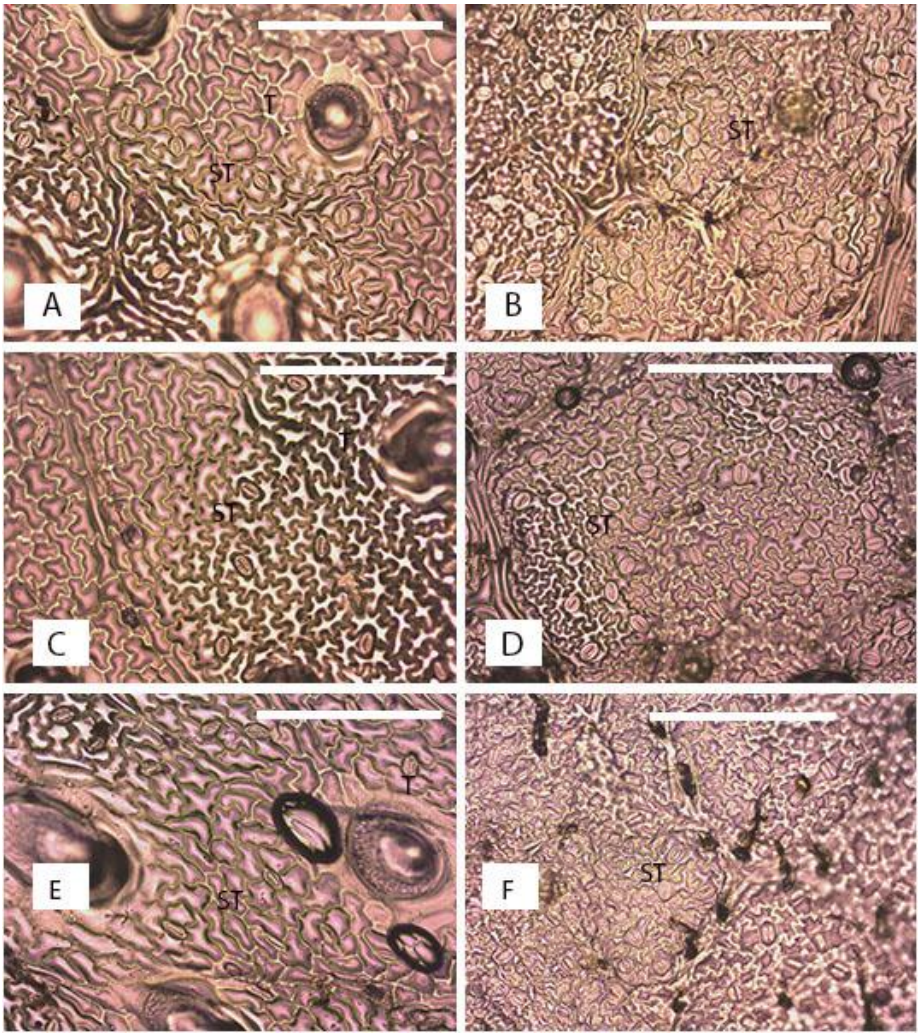

Fig. 4: Photomicrographies of upper and lower epidermal tissue of three Jerusalem artichoke cultivars, A: upper epidermis of Alba cultivar and B: lower epidermis of Alba cultivar, C: upper epidermis of Balady cultivar and D: lower epidermis of Balady cultivar, E: upper epidermis of Fuza cultivar and F: lower epidermis of Fuza cultivar, ST: Stomata, T: trichomes (Bars: 100 µm). Slides of stomata were prepared and examined using light microscope (Zeiss Axioscope 2+; Zeiss International, Oberkochen, Ostalbkreis, Germany) and photographed using Scope-Photo software ((Scopetek, München, Germany) by Neama Abdalla.

Table 3: Number of stomata of upper and lower epidermis of three Jerusalem artichoke cultivars.

	No. of stomata/250 µm^2	
Cultivars	Upper epidermis	Lower epidermis
Alba	14.2±0.02	43.6±05
Balady	12.5±0.05	42.8±0.03
Fuza	10.01±0.01	38.00±0.10
F test	**	**
$LSD_{0.05}$	0.085	0.17

4. Conclusions

Stem and leaf anatomical differences among the three tested cultivars of Jerusalem artichoke (Alba, Balady and Fuza) were evaluated. The anatomical differences mainly depend on the genetic structure of each cultivar. The highest main values of stem anatomical parameter as well as vascular tissues (xylem and phloem), thickness and xylem vessels diameter were recorded in Balady and Fuza cultivars. While the highest main values of leaf anatomical parameters as well as mesophyll tissue (palisade and spongy tissues) thickness and the density of stomata in the upper and lower epidermis were obtained in Alba and Balady cultivars. This study may recommend carrying furthermore studies on cultivation of JA under different environmental conditions and may also help in expecting the changes in the anatomy of this plant under biotic and abiotic stresses.

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5. Refereneces

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