

LEAF MICROMORPHOLOGY OF JUTE (*CORCHORUS OLITORIUS* L.) IN CONDITIONS FROM CLUJ COUNTY

Rodica VÂRBAN, Dan VÂRBAN, Ioana CRIȘAN

University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca,
Faculty of Agriculture, 3-5 Calea Manastur, 400372, Cluj-Napoca, Romania

Corresponding author email: dan.varban@usamvcluj.ro

Abstract

With a long history of cultivation in warm climates, Corchorus olitorius is a versatile plant with many utilizations. This species has been used for fibres, as leafy vegetable, for medicinal purposes, has phytoremediation potential and could present interest for a variety of emerging applications from agriculture to biotechnology. The aim of this research was to provide a micro-morphological characterization for leaves of jute plants grown for the first time in conditions from Cluj county, Romania. Microscopic analysis revealed that jute leaves displayed paracytic stomatal complexes, average leaf stomata density of 518.31 per mm², and characteristic capitate glandular trichomes less than 50 μm in size. Leaf insertion exercised a significant influence on stomata density, with an increased stomata density in leaves from secondary branches compared to leaves from first order stems, fact that is consistent with Zalenski's law. Plants developed a bushy habitus in the field and produced flowers and fruits towards the end of the summer. It is proposed that C. olitorius to be introduced in cultivation in local conditions for culinary and medicinal purposes.

Key words: stomata density, trichome, leaf, abaxial, food, utilization.

INTRODUCTION

Jute belongs to genus *Corchorus* from family *Malvaceae*, subfamily *Grewioideae* (Benor, 2018). *Corchorus olitorius* is one of the most cultivated species from this genus and is originally from Africa (Benor, 2018; Mukul, 2020). *C. olitorius* has a variety of utilizations and potential applications. There are two cultivar groups within *C. olitorius*: Olitorius and Textilis. First group has small habitus and is used as vegetable, while second group grows up to 4 m in height and is farmed for fibres (<https://pfaf.org>). Currently, jute is cultivated throughout the tropical and subtropical regions of the world: Asia, Africa, Central and South America (Mukul, 2020), where is recognized for the culinary and medicinal purposes and it is commonly consumed as leafy vegetable (Traoré et al., 2019; Chigurupati et al., 2020; Samuel et al., 2020). Also, jute occupies second place after cotton as most important fibrous plant crop in the world (Mukul, 2020). Jute has been used traditionally for ropes and hessian fabrics. Due to the remarkable durability of jute bast fibres, these have started to be used in automobile industry at lining the interior panels and trucks as well as for clothing the seat backs

(Chandekar et al., 2020). Recent advancements indicate that jute polymer composites have the potential to further extend the utilization of these fibres due to improved properties with promising opportunities particularly for constructions industry among others (Chandekar et al., 2020). The plant was shown to be host for many useful endophytic microorganisms that can find applications in agriculture or biotechnologies (Ahmed et al., 2016; Das et al., 2017; Haidar et al., 2018). The wide possibilities for utilization explain the expansion of jute crops.

Most important physiological process that sustains the life of plants is photosynthesis. This process relies on gas exchange that is mediated by small openings on the leaf surface called stomata (Xiong and Flexas, 2020). The patterning of leaf surface with stomata is a complex process controlled by genetic mechanisms (Baillie and Fleming, 2019), and strongly influenced by environmental factors (Vesely et al., 2020). Studies on stomata parameters have been considered relevant for characterization of genotypes (Miljkovi et al., 2013), were proposed as selection criteria in plant breeding programs for the identification of drought resistant genotypes (Li et al., 2017),

have been used to study adaptability of plants across altitudinal gradients (Holland and Richardson, 2009), acclimatization (Rodrigues et al., 2020), or were put in relationship with various treatments, such as inoculation with beneficial microorganisms (Chitarra et al., 2016). As key structures that are in relationship both with assimilation and transpiration rate (Xiong and Flexas, 2020), stomata parameters are important for the characterization of plants and their adaptability to the environmental conditions. The study of the stomata parameters such as density could reveal important information about species proposed to be introduced into cultivation in non-native geographical regions.

The aim of this research was to screen the stomata density of *Corchorus olitorius* plants grown in conditions from Cluj County Romania. The research had two objectives: 1) analysis of leaf micro-morphology, 2) identification of the histological gradient for leaf stomata density in jute plants.

MATERIALS AND METHODS

Corchorus olitorius L. plants from this study were obtained from seeds belonging to two accessions: XX-0-GZU-07100734, XX-0-CLA-4158, provided through International Plant Exchange Network. Seeds were sown on 13th March 2019 in pots with eutrophic peat, in greenhouse. Seeds sprouted on 25th March 2019 and seedlings were replanted in larger pots on 5th April 2019. Because in the month of May were heavy rains, the obtained jute plants were transplanted in field conditions from Agro-Botanical Garden of USAMV Cluj-Napoca on 2nd June 2019. Distance between rows was 50 cm and between plants per row 40 cm. Plants were watered until became established. Weed control was performed mechanically and manually. Soil in the Agro-Botanical Garden has clay-loam type, neutral pH, low humus level, good nitrogen, phosphorus, and potassium content (Crişan et al., 2018).

Samples for microscopic analysis were collected on 3rd September 2019. Leaves chosen had roughly similar size. Imprint samples collection and analysis was conducted following similar procedure as Crişan et al.

(2018). Observation was performed on abaxial surface imprints under Bresser Biolux NV microscope with camera. In total were analysed under microscope 48 leaf imprints (6 plants × 8 leaves, where 4 leaves from first order stems and 4 leaves from second order branches of each plant). Half of the analysed samples belonged to first accession and the other half belonged to the second accession. Stomata density was determined on 240 fields of view, corresponding to 5 fields of view analysed per leaf sample. Field of view at 640× magnification had Ø 0.235 mm, measured with stage micrometre. Density of stomata determined corresponded to $\pi r^2 = 0.04337 \text{ mm}^2$ and then calculated for 1 mm^2 . Stomata were counted only for the areas between leaf veins and marginal stomata were considered in the field of view only if whole ostiole was visible. Images were taken using Photomizer 2 Bresser edition software and further processed with ArcSoft Photo Impressions 5 software. Statistical analysis was conducted with Origin software by OriginLab.

RESULTS AND DISCUSSIONS

Climatic data from the experimental year are presented in Figure 1.

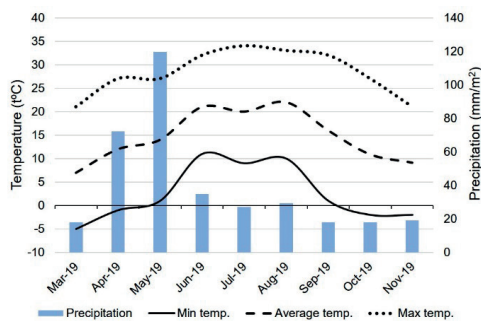


Figure 1. Climatic data for interval March-November 2019 in Cluj-Napoca, Romania (<https://en.tutiempo.net>, <https://www.wunderground.com>)

During 2019 in Cluj-Napoca, the last spring month with frost was April while highest precipitation levels were registered in May, before planting the jute plants in the field. Maximum temperatures exceeded 30°C for the months of June-September, and the highest temperature (of 34°C) occurred in July. In

October, the temperatures dropped below 0°C, marking the end of vegetative season. The accessions developed in Agro-Botanical Garden for a duration of 4 months (June-September), were healthy and displayed a bushy-habit. The flowering and fruit development occurred towards the end of summer and beginning of September.

Microscopic analysis of leaf imprint samples revealed the leaf surface micromorphology. It was determined that *Corchorus olitorius* accessions presented paracytic stomata complexes, because stoma was surrounded by two subsidiary cells that were parallel to the longitudinal axes of the pore and guard cell

pairs (Figures 2e, 2f). Leaf surface presented characteristic capitate hairs having <50 µm in length. These trichomes presented a basal cell, a short unicellular stalk and enlarged tip, aspect that suggests to the secretory function as glandular trichomes (Figures 2c, 2d). Epidermal cells were preponderantly isodiametric (Figures 2e, 2f). Analysis of variance revealed that leaf insertion had a highly significant influence on leaf stomata density ($p < 0.001$), explaining 79% of variance registered by this parameter. Average stomata density on first order stems was 463.26 per mm² and increased to an average of 573.36 per mm² on leaves of second order branches (Tables 1 and 2).

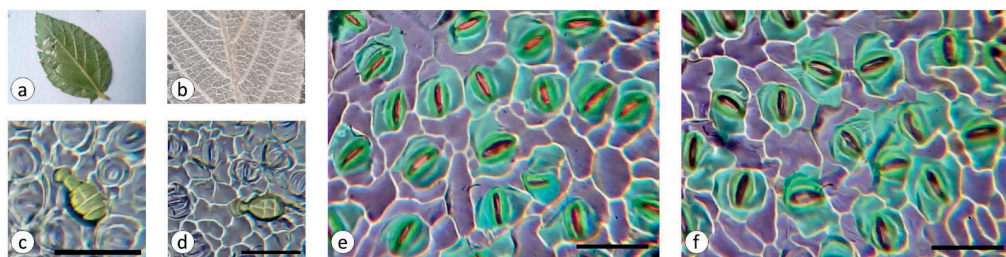


Figure 2. *Corchorus olitorius* samples: a) leaf with applied polish; b) abaxial leaf imprint; c), d) capitate glandular trichomes highlight in yellow; e), f) microscopic observation of stomata, where: red - stoma, green - guard cells, light blue - subsidiary cells, purple - epidermal cells, magnified 640×, bar = 50 µm (Original)

Table 1. Influence of leaf insertion and individual plant on abaxial stomata density of *Corchorus olitorius*

Factors	F value	p value	Significance	η ²
Leaf insertion (L)	47.968	<0.001	***	0.79
Plant (P)	1.005	0.368	n.s.	0.03
Interaction (L × P)	5.486	0.005	**	0.18

Note: Two-way ANOVA, $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) ; η² - partitioning of variance

Table 2. Stomata density variation according to leaf insertion in *Corchorus olitorius*

Leaf insertion	Mean per mm ²	±SD	±SE	Variance (s ²)
1 st order stems	463.26	93.24	8.51	8693.91
2 nd order branches	573.36	150.91	13.78	22775.22
Overall	518.31	136.79	8.83	18711.87

Note: standard deviation (SD) and standard error of mean (SE)

This gradient corresponds to an increase of stomata density according to leaf insertion, higher stomata density being associated with leaves from higher order branches. However, one can notice that higher stomata density variability among fields of view analysed were present on samples from second order branches compared to first order stems (Table 2). There

was no significant difference among individual plants regarding stomata density ($p = 0.368$) (Table 1), thus the overall average stomata density of 518.31 per mm² determined in this study can be considered representative for these accessions in local conditions. Frequency histogram revealed that on the first order stems most leaf samples had a stomata density

between 400-500 per mm^2 while for second order branches most samples had a stomata density between 500-600 per mm^2 . It can also be observed that leaves from higher order branches had lower densities of stomata ($<500/\text{mm}^2$) as well as samples with densities exceeding 600 per mm^2 (Figure 3).

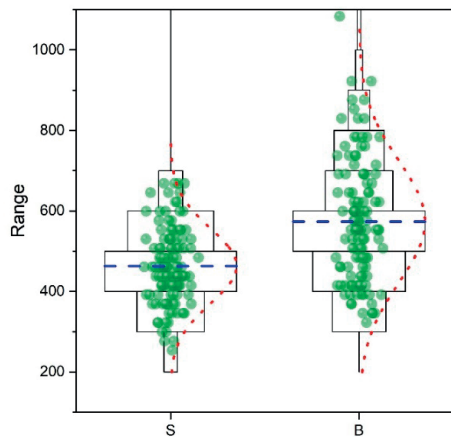


Figure 3. Frequency histogram showing classes for leaf stomata density (number per mm^2) in *Corchorus olitorius*: first order stems (S), second order branches (B), blue dash line - average, red dotted line - normal distribution curve.

Similar with the observation from this study, Ghosh et al. (2004), also described stomata complexes in *Corchorus olitorius* as paracytic. As for stomata density obtained in Cluj conditions, the abaxial surface average is comparable to the abaxial density of 497.70 per mm^2 reported for *Corchorus olitorius* in conditions from India (Maity and Datta, 2011). However, according to data from literature, stomata density can vary widely between *Corchorus* species and hybrids. Abaxial stomatal densities between 300-400 per mm^2 were identified in species: *C. pseudocapsularis*, *C. trilocularis*, *C. fascicularis*, *C. pseudo-olitorius*, *C. tridens*, between 500-600 per mm^2 in *C. trilocularis* \times *C. capsularis*, *C. capsularis* and high stomatal densities of over 600 and respectively 700 per mm^2 in *C. urticaefolius* and *C. aestuans* (Maity and Datta, 2010; 2011). Compared to these species, *Corchorus olitorius* stomata density situates within a middle range. Because the stomata distribution in *Corchorus olitorius* is amphistomatic and anisostomatic

(Ghosh et al., 2004), the measurements of stomatal parameters on abaxial surface are highly relevant from physiological perspective. The rate at which stomata facilitates gas exchange is determined by stomata density as well as size (Baillie and Fleming, 2019), indicating to the importance of this leaf micromorphological parameter. Previous research in various species, revealed that insertion gradients influence stomata densities, with an increase from bottom to middle insertion levels. This histological gradient for stomata parameters is explained through Zalenksi's law as a function of distance from the root (Sesták, 1985). In Cluj conditions, *Corchorus olitorius* presented an increase of stomata density from first order stems to secondary branches. Trichomes identified on the leaves of *Corchorus olitorius* from this study resemble those described in other species from family *Malvaceae*. Study on several *Hibiscus* species from Pakistan, has put in evidence in all taxa studied the presence of glandular capitate trichomes with unicellular stalk and multicellular uniseriate or biseriate head, with length up to 50 μm (Shaheen et al., 2009). A few other trichome morphologies described by the cited authors were identified in same *Hibiscus* species but such morphologies were not observed in *Corchorus olitorius* leaves from this study. Leaf micromorphological studies revealed the presence of capitate glandular trichomes in *Malva sylvestris* (Romitelli and Martins, 2013), as well as in *Theobroma* spp. (Garcia et al., 2014), that are also from family *Malvaceae*. Glandular trichomes occur repeatedly across various plant families and species, and their morphology is related to the type of metabolites they secrete. Capitate trichomes usually are associated with non-volatile compounds, that are released directly at the surface by the cells from the tip. This is a major difference compared to volatile producing-trichomes, that present a storage space that might be subcuticular or between secretory cells, while the compounds get released only when trichomes are damaged (Schuurink and Tissier, 2020).

Latest insights related to the potential use of *Corchorus olitorius* plants, as well as many emerging applications (Table 3), are justifying

the revived interest for the study and cultivation of this plant. From this perspective future efforts should be directed towards testing and monitoring acclimatization of genotypes as

well as defying the most suitable utilizations based on characterization of the locally grown plants.

Table 3. Recent insights related to applications and potential utilization of *Corchorus olitorius*

Importance	Findings	Sources
fibres and fabrics	Jute fibres can be used as sound absorber material efficient above 3500 Hz frequency.	Sengupta et al., 2021
	Jute fibre reinforced composites present opportunities for wider utilizations due to improved properties.	Chandekar et al., 2020
	Plant breeding selection schemes for improved fibre production can be based on anatomical study of stems.	Mukul et al., 2020
	Jute fibre bonded with polyvinyl alcohol can produce a highly functional non-woven fabric.	Sengupta et al., 2020
food	Fruits soup showed good nutritional value and acceptable sensory characteristics.	Samuel et al., 2020
	Leaves showed significant decrease of β -carotene following cooking. Optimizing the cooking method is important.	Traoré et al., 2019
medicinal	Liposomes proved entrapment efficiency >80% for phytol extracted from leaves, enhancing delivery and pharmacological activity.	Mohammad et al., 2021
	Leaves ethanolic extract demonstrated antioxidant and anti-diabetic effect.	Chigurupati et al., 2020
	Hydroalcoholic extract of leaves showed neuromodulator potential.	Wagdy et al., 2019
corrosion inhibitor	Plant stem extract is inhibitor of mild steel corrosion to H ₂ SO ₄ solution, with up to 93% efficiency.	Gobara et al., 2017
biosynthesis of Au and Fe nanoparticles	70% aqueous ethanolic leaves extract reduced gold aureate and ferric chloride solutions with formation of gold and iron oxide nanoparticles in one step.	El-Rafie et al., 2016
phytoremediation	Plants survived concentration of Cd 20 mg/kg in soil; translocation factor was >1 regardless of concentration.	Ndlovu et al., 2020
	Absorbent obtained from jute stick charcoal was demonstrated to have potential for dye removal from water.	Chakraborty et al., 2020
anti Cu-stress treatment	Jute seeds extract demonstrated protective effect against copper stress in tomato seedlings.	İşeri et al., 2018
useful endophytes	Out of the 27 bacteria species isolated from various plant organs, some demonstrated plant growth enhancing capabilities.	Haidar et al., 2018
	<i>Grammothele lineata</i> isolated from jute plant produced taxol that exhibited <i>in vitro</i> cytotoxic affect against HeLa cancer line.	Das et al., 2017
	<i>Aspergillus terreus</i> KP900973 isolated from jute stem produced xylanase enzyme, that could be used as dough-raising agent.	Ahmed et al., 2016

It is proposed that in local temperate-continental conditions of Cluj-Napoca, acclimatization and cultivation of *Corchorus olitorius* may present increased interest particularly due to the culinary and medicinal value of these plants.

CONCLUSIONS

This research provides a leaf micro-morphologic characterization of jute plants (*Corchorus olitorius*) grown for the first time in conditions from Cluj county, Romania. Results indicate that in local conditions, jute plants can develop in field for at least four months, reaching bloom and producing fruits.

Plants had an average stomata density of 518.31 per mm², comparable with densities reported in conditions from India where jute is well established in cultivation.

It is proposed that this species can be grown in local conditions for culinary and medicinal purposes.

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