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MORPHOLOGICAL AND ANATOMICAL CHARACTERIZATION OF SAFFLOWER (*Carthamus tinctorius* L.) HYPSOPHYLS AND LEAVES

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Abstract

Safflower (Carthamus tinctorius L.) is an annual plant that belongs to the Asteraceae family. It is drought resistant and both seeds and flowers have multiple uses in food, pharmaceutical, dermato-cosmetic and textile industries. Safflower can also be grown as an ornamental plant. The main objective of this study is to identify the morphological and anatomical differentiation of safflower hypsophylls and leaves. The study was conducted on two varieties of C. tinctorius grown in the Botanical Garden of the U.S.A.M.V. Bucharest. The samples were examined with Leica stereomicroscope, Leica optical microscope and scanning electron microscope. The results showed differences between the two varieties regarding the shape of the blade, the arrangement of the vein, the presence and arrangement of the secretory channels. This result brings new elements in the identification of bioactive compounds and for the differentiation of the varieties.

Key words*: safflower, secretory channels, leaves, morphology.*

INTRODUCTION

Oilseed crops have an important role not only for their nutritional value and therapeutic properties but also for industrial uses.

With global demand, there is a need to develop varieties that have higher oil content, higher productivity, and are resistant to different challenging environments (Weselake, 2016; Zafar et al., 2019; Kotecka-Majchrzak et al., 2020; Hassani et al., 2020).

In general, most lipids are stored in the seeds of oil crops in the form of high-energy-density triacylglyceride (TAG), but there are studies that show their presence in important quantities in non-seed organs, including mesocarp, flower petals, pollen, stems, tubers, and seed pods, husks and also in the leaves (Alameldin et al., 2017; Nikiforidis, 2019; Vanhercke et al., 2019; Zafar et al., 2019).

In the leaves, TAG synthesis and oil bodies formation can be influenced by various stress factors like darkness, extreme temperature (heat, cold, freezing) and senescence (Weber et al., 2018; Nikiforidis, 2019; Vries & Ischebeck, 2020).

Fungal infections of the leaves can also affect TAG content by increasing linolenoyl content. In senescent leaves, oil bodies have antifungal properties, producing the antifungal compound 2-HOT. Thus, leaf oil cavities may have an intercellular role to produce lipid compounds for plant defence responses (Vanhercke et al., 2019; ENV/JM/MONO 2020).

Nikiforidis et al. (2019) have shown that oil bodies are more present in senescent leaves than mature leaves and the genes involved in the biosynthesis of oleosin are not found in the leaves, so they suggested that the components of oil bodies from the seeds are different than those from the leaves.

Different accumulation of lipids can lead to some clues that can be useful in strategies for increasing oil content in plant stems and leaves. Different extraction methods can be applied for the extraction of oil from vegetative tissues with importance in biodiesel and pharmaceutical industry (Vanhercke et al., 2019; Singh et al., 2021).

Secretory structures of oil bodies can also be found as secretory cavities and ducts in leaves, stems, roots and flower. They are important taxonomic characters for many plant families (Cury & Appezzato-da-Glória, 2009; Fernandes et al., 2018)

Asteraceae family has secretory cavities formed in a schizogenic way that elongate and give the impression of secretory channels (Bartoli et al., 2011; Cury & Appezzato-da-Glória, 2009; Russin et al., 1992)

A well-known oilseed and medicinal plant belonging to *Asteraceae* family is *Chartamus tinctorius.*

Leaf essential oil such as methyl eugenol, α-pinene, cinnamyl acetate, flavonoids like luteolin and its glucopyranosides, carotenoids, dehydroabietylamine, have also been found in the leaves, having antibacterial, antifungal, antiinflamatory, antioxidative, hepatoprotective activity (Lee 2002; Asgarpanah, 2013; Salem et al., 2014). The leaves can be also used in: vitiligo, psoriasis, pain relief according to Delshad et al. (2018). Safflower young leaves can be used as a vegetable food, and leaves and stems can be used as green fodder, hay or silage (Heuzé & Tran, 2015; Dobrin et al., 2016).

In the growth and development stages, young leaves form a rosette, followed by rapid stem elongation, branching, then flowering, with alternate leaves on each side of the stem (Dajue & Mündel, 1996). Leaf size may vary according to variety and position on the plant, ranging between 2.5-5 cm wide and 10-15 cm long. The leaf can be sessile and ovate-lanceolate. In some varieties, upper leaves often develop spines, while lower on the stem are usually spineless (Dobrin & Marin, 2015).

Morphological characteristics regarding the presence or absence of leaf spines are related to the fertility of the plant so the leaves of fertile plants have spines, and non-spiny traits are associated with sterile plants. Knowing safflower leaves botanical and morphological characteristics is important in the selection and production of pure hybrid seeds and the faster breeding of elite varieties (ENV/JM/MONO 2020).

The main objective of this study is to identify the botanical and morphological aspects of distiction of the senescent hypsophylls and leaves of two varieties of *Carthamus tinctorius* L., and characterisation of the secretory structures of oils, in order to differentiate the varieties and for the possible standardization with importance in the botany, agriculture and pharmaceutical industry.

MATERIALS AND METHODS

The safflower varieties used in the study were: a spineless variety (Zanzibar) with a high content in linoleic acid (Dobrin & Marin, 2015) and a variety with spines (S2). The experiment was carried out in the Botanical Garden of the U.A.S.V.M. Bucharest. The morphological and anatomical characterisation of safflower hypsophylls and leaves was performed in the Research Center for Studies of Food Quality and Agricultural Products, U.A.S.V.M. Bucharest. The hypsophylls and leaf samples were harvested in September. The samples were rehydrated and stored in 70% ethanol (Talbot & White, 2013) before being analysed with a Leica S8 APO stereomicroscope, optic microscope Leica DM 1000 LED and with the Scanning Electron Microscope (SEM) FEI Inspect S50.

RESULTS AND DISCUSSIONS

Hypsophylls anatomy and morphology

The presence of hypsophylls was observed in both varieties. For the anatomy and morphology characterisation the hypsophylls were examined using stereo-microscope, optic microscope and SEM.

Figure 1. Stereo microscope images of hypsophylls of *C. tinctorius* var. Zanzibar and S2

Zanzibar variety has hypsophylls lamina ovate to obovate shape, with round apex, slightly narrowed to the base, cross-venulate; the margins are whole, with a smooth margin, the blade is smooth and shiny, without spines (Figure 1 A).

The hypsophylls from variety with spines (S2) have lanceolate lamina shape, with acuminate apex; cross-venulate, slightly denticulate, with mate blade and few spines (Figure 1B).

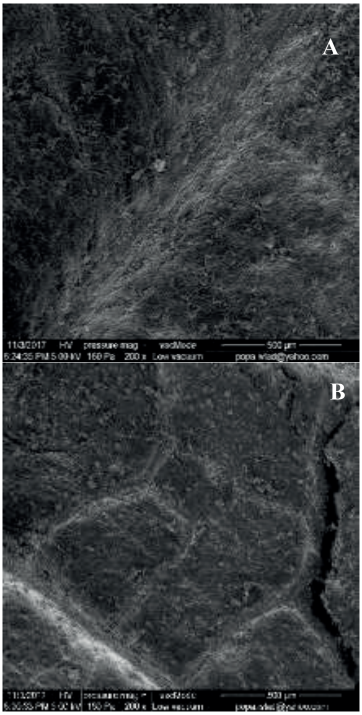

Figure 2. Hypsophylls surface from *C. tinctorius* var. Zanzibar (A) and S2 (B) examined with SEM

We found open and closed stomata on senescence hypsophylls surfaces of both safflower varieties, on low vacuum SEM, at 500 µm (Figure 2 A and B).

In the Figure 3 and 4 it can be seen that the structures identified do not have the regular shape of secretory channels, but are spaces that develop in the mesophyll that accompany the secondary or tertiary conducting vessels. Also, it can be seen that the colour of the Zanzibar variety`s structures are orange to red (Figure 3), and of S2 variety are yellow (Figure 4).

The presence of oil cells in *Asteraceae* has been identified in the foliar structure also by Rikisahedew & Naidoo (2018).

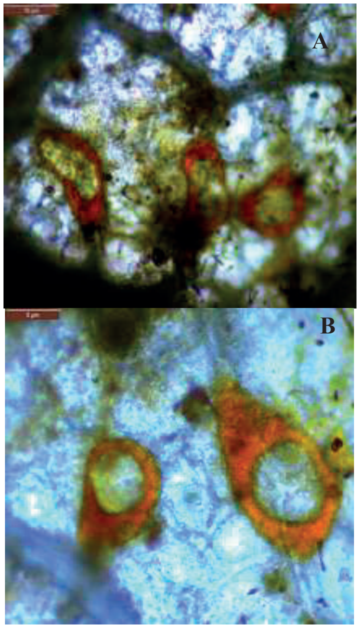

Figure 3. Hypsophylls from *C. tinctorius* var. Zanzibar-middle area 10x, scale bar =10 µm (A), hypsophyll detail 20x, scale bar =5 µm (B)

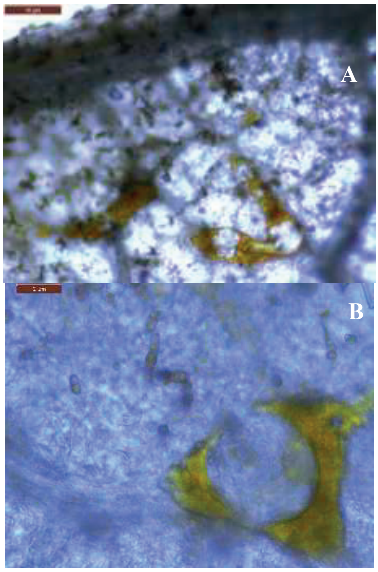

Figure 4. Hypsophylls from *C. tinctorius* var. S2 -middle area 10x, scale bar =10 µm (A), hypsophyll detail 40x, scale bar =2 µm (B)

Leaves anatomy and morphology

Leaves anatomy and morphology were examined with stereo-microscope, optic microscope and SEM.

Figure 5. *C. tinctorius* var. Zanzibar leaf apex (A) and detail of leaf (B) -stereo microscope images

In the Figure 5 A and B it can be seen that Zanzibar variety`s leaves, like hypsophylls, has elliptic-cuneate shape, and are cross-venulate; the margins are entire, with a smooth margin, smooth and shiny blade, without spines, like hypsophylls.
The leaves from variety with spines have lanceolate lamina shape, with acuminate apex, and cross-venulate, slightly denticulate, mate blade, with spines, like hypsophylls (Figure 6 A and B).

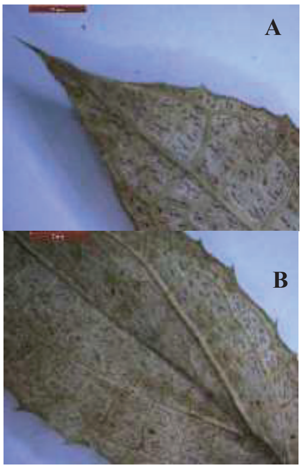

Figure 6. *C. tinctorius* var.S2 leaf apex (A) and detail of leaf (B) -stereo microscope images

On low vacuum SEM at 300 µm and 500 µm images of the senescence leaves surfaces, open and closed stomata (Figure 7 A and B) were found on both safflower varieties, like in hypsophylls.

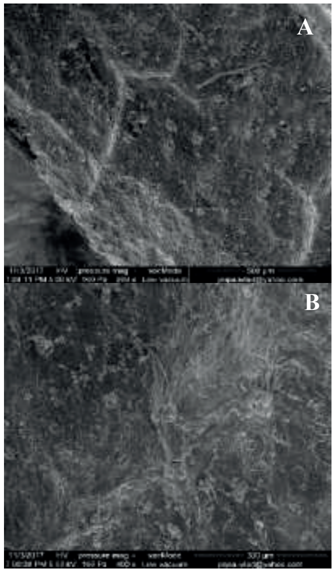

Figure 7. Leave surface from *C. tinctorius* var. Zanzibar (A) and S2 (B) examined with SEM

It can be seen that in the leaf, the colour of the Zanzibar variety`s structures (Figure 8 A and B), is similar with that of S2 variety (Figure 9 A and B).

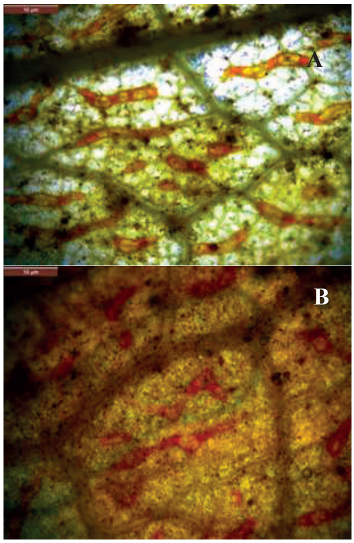

Figure 8. Leaf lamina from *C. tinctorius* var. Zanzibar-middle area 10x, scale bar =10 µm (A), leaf lamina base detail 10x, scale bar =10 µm (B)

In figures 8 and 9 it can be noticed that the structures identified in the leaves do not have the regular shape of secretory channels, but are spaces that develop in the mesophyll that accompany the secondary or tertiary conducting vessels, like in hypsophylls (Figure 3 and 4).

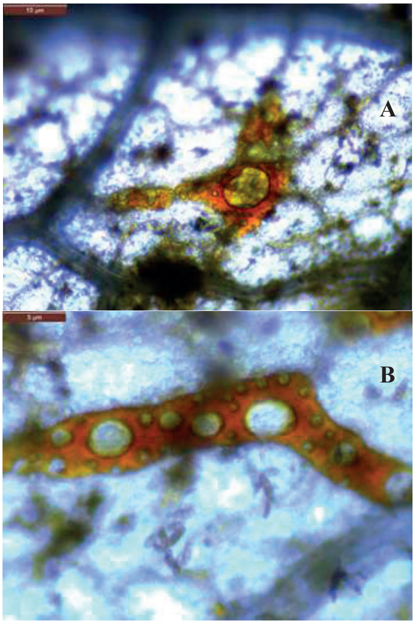

Figure 9. Leaf lamina from *C. tinctorius* var. S2 -middle area 10x, scale bar =10 μm (A), middle area 20x, scale bar =5 μm (B)

CONCLUSIONS

The originality of the paper presented, in terms of anatomical descriptions, is that it comes and confirms the following observation: plants in the *Asteraceae* family have secretory cavities formed in a schizogenic way that elongate and give the impression of secretory channels.

The description of these cavities should not be based only on cross sections (according to Lestern & Curtis, 1989).

In the presented images it can be seen that these structures do not have the regular shape of secretory channels, but are spaces that develop in the mesophyll that accompany the secondary or tertiary conducting vessels.

There are differences between the two varieties of *Carthamus tictorius* L. regarding the shape of hypsophylls and leaves blade, the arrangement of the vein, the presence, arrangement and irregular shape of the secretory cavities.

Macro and microscopic identification of the morphological and anatomical characteristics of safflower senescence hypsophylls and leaves brings new elements for the differentiation of varieties, for the possible standardization and in the identification of bioactive compounds, with importance in the botany, agriculture and pharmaceutical industry.

REFERENCES

Alameldin, H., Izadi-Darbandi, A., Smith, S.A., Balan, V., Jones A.D., Sticklen, M. (2017). Production of Seed-Like Storage Lipids and Increase in Oil Bodies in Corn (Maize; *Zea mays* L.) Vegetative Biomass, *Industrial Crops and Products*, *108*. 526–534.

Asgarpanah, J., Kazemivash, N. (2013). Phytochemistry, pharmacology and medicinal properties of *Carthamus tinctorius* L., *Chin J Integr Med*., *19*(2),153–159.

Bartoli, A., Galati, B.G., Tortosa, R.D. (2011). Anatomical studies of the secretory structures: Glandular trichomes and ducts, in *Grindelia pulchella* Dunal (*Astereae*, *Asteraceae*). *Flora - Morphology, Distribution, Functional Ecology of Plants, 206*(12), 1063–1068.

Cury, G., Appezzato-da-Glória, B. (2009). Internal secretory spaces in thickened underground systems of *Asteraceae* species. *Australian Journal of Botany*, *57*. 229–239.

Dajue, L., Mündel, H.H. (1996). Safflower. *Carthamus tinctorius* L. Promoting the conservation and use of underutilized and neglected crops. 7. Institute of Plant Genetics and Crop Plant Research, Gatersleben/ International Plant Genetic Resources Institute, Rome, Italy.

Delshad, E., Yousefi, M., Sasannezhad, P., Rakhshandeh, H., Ayati, Z. (2018). Medical uses of *Carthamus tinctorius* L. (Safflower): a comprehensive review from Traditional Medicine to Modern Medicine. *Electronic physician*, *10*(4), 6672–6681.

Dobrin, A., Marin, D. (2015) Research on safflower (*Carthamus tinctorius* L.) crop in the conditions of southeastern Romania. *Scientific Papers. Series A. Agronomy*, *LVIII*, 181–184.

Dobrin, A., Bădulescu, L., Dobrescu, A. (2016) The influence of sowing time and fertilizer levels on biomass yield of safflower crop from Southeastern Romania. *Journal of Horticulture, Forestry and Biotechnology*, *20*(4), 5–11.

Fernandes, V.F., Thadeo, M., Dalvi, V.C., Marquete, R., Xavier de Brito Silva, J., de Jesus Pereira, L., Meira, R.M.S.A. (2018). How to distinguish cavities from ducts in Casearia Jacq. (*Salicaceae*): Anatomical characterization and distribution, *Flora*, *240*, 89–97.

Hassani, S.M.R., Talebi, R., Pourdad, S.S., Naji, A.M., Fayaz F. (2020). Morphological description, genetic diversity and population structure of safflower (*Carthamus tinctorius* L.) mini core collection using

SRAP and SSR markers. *Biotechnology & Biotechnological Equipment*, *34*(1), 1043–1055.

Heuzé, V., Tran, G. (2015). Safflower (*Carthamus tinctorius*) forage. Feedipedia, a programme by INRAE, CIRAD, AFZ and FAO. https://www.feedipedia.org/node/173.

Kotecka-Majchrzak, K., Sumara, A., Fornal E., Montowska M. (2020). Oilseed proteins – Properties and application as a food ingredient. *Trends in Food Science & Technology, 106*, 160–170.

Lee, J.Y, Chang, EJ, Kim, HJ, Park, JH, Choi, SW. (2020). Antioxidative flavonoids from leaves of *Carthamus tinctorius*. *Arch Pharm Res*., Jun; *25*(3), 313–319.

Lersten, NR, Curtis, JD. (1989). Foliar oil reservoir and distribution in *Solidago canadensis* (*Asteraceae*, tribe *Astereae*). *Nordic Journal of Botany, 9*. 281–287.

Nikiforidis, C. V. (2019) Structure and functions of oleosomes (oil bodies). *Advances in Colloid and Interface Science*, *274*. 102039.

Rikisahedew, J.J., Naidoo, Y. (2018). Phyto-histochemical evaluation and characterisation of the foliar structures of *Tagetes minuta* L. (*Asteraceae*). *South African Journal of Botany*, *115*. 328–329.

Russin, W.A., Uchytil, T.F., Durbin, R.D. (1992). Isolation of structurally intact secretory cavities from leaves of African marigold, *Tagetes erecta* L. (*Asteraceae*). *Plant Science*, *85*(1), 115–119.

Salem, N., Msaada, K., Hammami, M., Selmi, S., Limam, F., Marzouk, B., (2014). Regional and phenological effects of Tunisian Safflower (*Carthamus tinctorius*) essential oil leaves. *Journal of New Sciences*, *9*(1).

Singh, R., Arora, A., Singh,V. (2021). Biodiesel from oil produced in vegetative tissues of biomass – A review. *Bioresource Technology*, *326*. 124772.

Talbot, M.J., White, R.G. (2013). Cell surface and cell outline imaging in plant tissues using the backscattered electron detector in a variable pressure scanning electron microscope. *Plant Methods*, *9*. 40.

Vanhercke, T., Dyer, J.M., Mullen, R.T., Kilaru, A., Rahman, M., Petrie, J.R., Green, A.G., Yurchenko, O., Singh, S.P. (2019). Metabolic engineering for enhanced oil in biomass. *Progress in Lipid Research*, *74*. 103–129.

Vries, J., Till Ischebeck, T. (2020). Ties between Stress and Lipid Droplets Pre-date Seeds. *Trends in Plant Science, 25*(*12*), 1203–1214.

Weselake, R.J. (2016). Chapter 15 - Engineering Oil Accumulation in Vegetative Tissue, Editor(s): Thomas A. McKeon, Douglas G. Hayes, David F. Hildebrand, Randall J. Weselake, Industrial Oil Crops. AOCS Press, 413–434.

Weber, K., Casali, C., Gaveglio, V., Pasquaré, S., Gómez, E.M., Parra, L., Erjavec, L., Perazzo, C., Tome, M.C.F. (2018). TAG synthesis and storage under osmotic stress. A requirement for preserving membrane homeostasis in renal cells. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, *1863*(9), 1108–1120.

Zafar, S., Li, Y.L., Li, N.N., Zhu, K.M., Tan X. L. (2019). Recent advances in enhancement of oil content in oilseed crops. *Journal of Biotechnology*, *301*. 35–44.

ENV/JM/MONO (2020)14-Consensus document on the biology of safflower (*Carthamus tinctorius* L.). *Series on Harmonisation of Regulatory Oversight in Biotechnology, 68*.