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MICRO-MORPHOLOGICAL FEATURES OF ACHENE OF WILD ANNUAL SUNFLOWERS

ABSTRACT: The aim of this research is to characterize wild annual sunflowers on the basis of achene micro-morphology. Plant material was grown up on an experimental field of the Institute of Field and Vegetable Crops in Novi Sad during 2015. Achene samples were hand-collected at the time of physiological maturity. Morphological measurements of achenes were performed using stereoscopic microscope Leica MZ16 with Leica DFC 320 Camera. The micro-morphological diversity of achenes was assessed using scanning electron microscopy (SEM). Obtained results indicated the presence of some quantitative and qualitative differences in achene characteristics among analyzed species, such as in their size, color, carpopodium and stylopodium shape, and distribution of trichomes on the achene surface. The carpopodium of examined species was asymmetrical at the maturity. Differences in the cuticle and wax ornamentation in different parts of the achenes, on the anticlinal walls of epidermal cells, were identified. The SEM analysis revealed the presence of non-glandular, multicellular bi-seriate trichomes (twin hairs) on the achene surface. This trichome type consisted of two elongated, parallel cells of different length. Considering the distribution of trichomes among the apical, median and basal regions of the fruit, most of the species demonstrated greater trichome density in the apical part.

KEYWORDS: achene, micro-morphology, trichomes

INTRODUCTION

The genus *Helianthus*, which belongs to Asteraceae family, is native to temperate North America and comprises 14 annual and 37 perennial species (Schilling 2006). Its botanical name *Helianthus* comes from the Greek words helios (sun) and anthos (flower). All annual *Helianthus* species, including cultivated sunflower, are diploid (2n = 34) (Kaya *et al.,* 2012).

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The fruit of Asteraceae is named achene, or cypsela, and is developed from bicarpellary ovary. In general, properties of reproductive structures are diagnostically important, and micro-morphological features of fruit surface are especially useful in taxonomy (Zarafshar *et al*., 2010). From taxonomical point of view, value of fruit micro-morphological features has already been recognized for many angiosperm families (Morozowska *et al*., 2012). Zhu *et al.* (2006) reported that a high diversity of fruit structures may provide some important insights into the phylogeny of different angiosperm groups. Anatomical characteristics of Asteraceae fruit have been described in scientific papers (Pandey 1989; Karanović *et al*., 2016), but rare data can be found on micro-morphological features of *Helianthus* fruit (Perez *et al*., 2006; Kocjan 2008). Trichomes are one of the most helpful micro-morphological characters which can be used in angiosperm taxonomy (Carlquist 1961). Also, in Asteraceae family trichomes morphology has already been used to clarify tribal and sectional classifications (Al-Shehbaz *et al*., 2006) and, more recently, it has been used as data in phylogenetic studies (Caruzo *et al*., 2011). The structure of trichomes is genetically controlled and, more or less, it is a stable taxonomic character. The role of non-glandular trichomes relies on their form, location on plant and direction or orientation. Usually, they provide protection, assist in pollen dispersal and reduce mechanical abrasion (Werker *et al*., 2000; Wagner *et al*., 2004). In Asteraceae family, in over 1,000 genera, there are many different types of trichomes (Panero and Funk 2008). The presence of twin or duplex achene hairs is one of characteristic features of the family (Metcalfe and Chalk 1950).

Nectaries occur on different plant organs and nectar is an attractive source of food for many species of flower visiting animals, particularly insects (Freitas *et al*., 2001). The topography, shape and anatomy of nectaries can be significantly different, even within one family (Petanidou *et al*., 2000). In Asteraceae, floral nectaries are annular and multicellular outgrowths at the top of the inferior ovary which surround the style base (Frei 1955).

Since there are only few reports on micro-morphological data of fruits in *Helianthus*, the aim of this research was to compare and characterize annual species of wild sunflower on the basis of morphological and micro-morphological features of their achenes.

MATERIAL AND METHODS

In this study five annual species of wild sunflower were analyzed: *H. annuus* L., *H. argophyllus* Torr. et A. Gray*, H. petiolaris* Nutt., *H. praecox* Engelm et A.Gray, and *H. debilis* Nutt. Mature achenes were collected from the experimental field of the Institute of Field and Vegetable Crops in Novi Sad during 2015 and examined for morphological and micro-morphological characters.

For examination, fifty randomly selected fruits per species were selected. Morphological measurements were performed using stereoscopic microscope Leica MZ16 with Leica DFC 320 camera. The following characters of achene

were studied: shape, surface, color, size (length and width), and also shape and position of carpopodium and stylopodium. Characteristics of achene trichomes, cuticle and wax ornamentation on the anticlinal walls of epidermal cells were not easily discerned using a light microscopy, so they were analyzed by SEM. For scanning electron microscopy, mature and dry achenes from each species were selected and directly mounted on a metallic stub using double adhesive tape and coated with gold for 180 s, at 30 mA (BAL-TEC SCD 005), and were subsequently viewed using JEOL JSM-6460LV electron microscope at 20 kV acceleration voltage.

RESULTS AND DISCUSSION

Most of the tribes of the family Asteraceae have quite similar cypsela features (Abid and Qaiser 2009), with the exception of *Helianthae*, *Eupatorieae* and *Inuleae*, where cypsela epidermal characteristics have been found very useful for tribal delimitation (Bremer 1994). In this study, the examined species shared many achene morphological properties when studied by stereoscopic microscope. Nevertheless, SEM showed that there were some micro-morphological differences. Achenes of all species were homomorphic, and obovate to widely obovate in shape (Figure 1, Table 1).

Table 1. Achene morphological characteristics of wild annual sunflowers.

Species	Shape of achene	Length of achene (mm)	Width of achene (mm)
H. annuus	wide obovate	4.5±0.41	2.3±0.29
H. argophyllus	narrow obovate	4.9±0.45	2.4±0.27
H. petiolaris	elongated	4.7±0.52	2±0.21
H. praecox	elongated	3.8±0.27	2±0.19
H. debilis	narrow obovate	4.4±0.39	2.98±0.32

The mature fruits of examined species were slightly different in size (Table 1). The color of sunflower fruits is determined by the presence or absence of pigments (Pandey 1989). For the examined species the basic color of the fruits was brown, but there were small variations in color. *H. annuus* achenes were golden-brown with two or more dark stripes in direction from base to achene apex. The stripes were variable in width from very narrow to very wide (Figure 1A). In *H. argophyllus* achenes were golden-brown in color with irregularly distributed dark patches (Figure 1B). *H. petiolaris* achenes were light gray with black patches (Figure 1C). *H. praecox* achenes were light brown with a lot of dark patches (Figure 1D) and in *H. debilis* their color was brown with irregularly distributed dark patches (Figure 1E). This variation of brown coloration between the species is correlated with different distribution of phytomelanin pigment in achene pericarp. According to Pandey (1989) phytomelanin in the pericarp of sunflower achenes is an effective mechanism for reducing yield losses due to achene injury by larvae of sunflower moth.

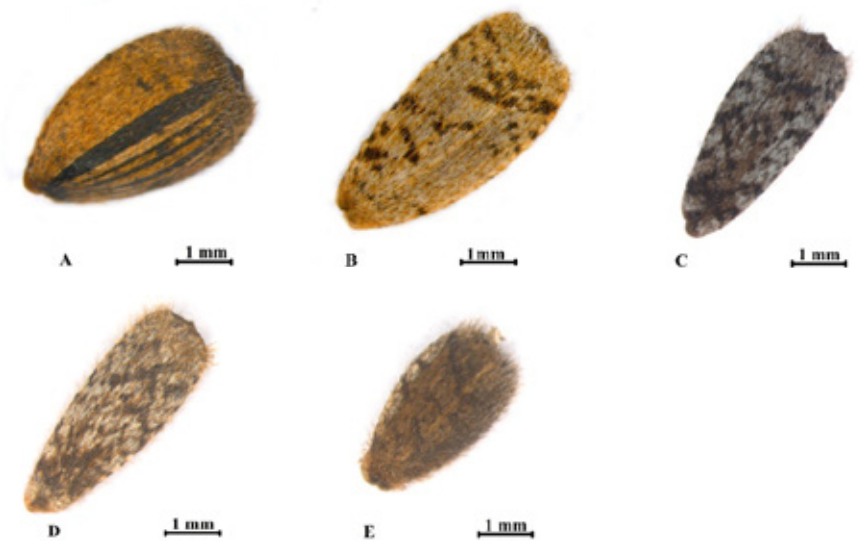

Figure 1. Light micrographs of achenes of wild annual sunflowers. A – *H. annuus*; B – *H. argophyllus*; C – *H. petiolaris*; D – *H. praecox*; E – *H. debilis*.

In all studied species, stylopodium was present at the upper part of achene (Figure 2C). The stylopodium was symmetrical and ring-like in all species. At the basal part of achene the carpopodium was distinct, asymmetrical and did not significantly differ in appearance among the species (Figure 2D). The largest dimensions of carpopodium and stylopodium were recorded in *H. annuus*, while the smallest were observed for *H. petiolaris*. In SEM micrographs of achenes, the cuticular ornamentation of the outer cell walls and the epicuticular wax were distinctly visible in all analyzed species (Figure 2). The fruit surface consisted of irregular epidermal cells which were parallel to the longitudinal fruit axis (Figure 2A and 2B).

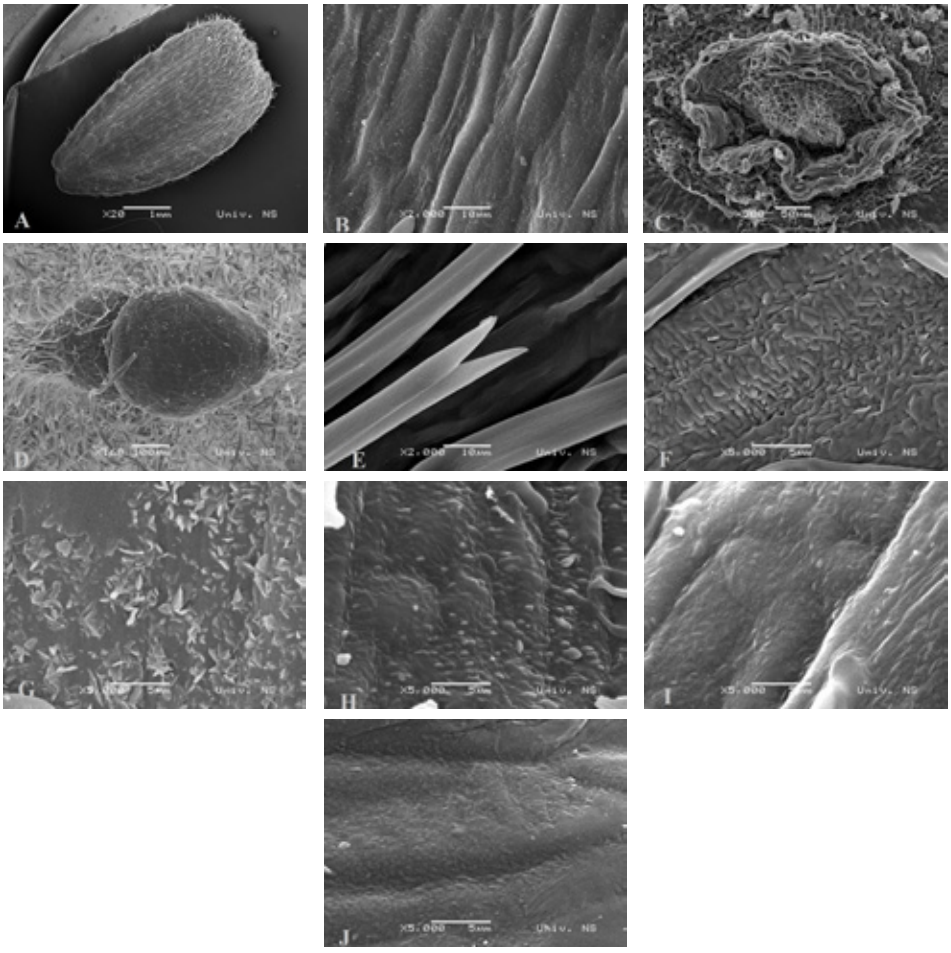

Figure 2. Scanning electron micrographs: A – mature achene (*H. argophyllus*); B – epidermal cells of achene (*H. annuus*); C – apical part of achene – stylopodium and nectariferouse tissue (*H. annuus*); D – basal part of achene – carpopodium (*H. debilis*); E – twin hairs with acute tip (*H. petiolaris*); F–J – epicuticular wax of pericarp (*H. argophyllus, H. debilis, H. annuus, H. praecox, H. petiolaris*).

All species have indistinct borders between epidermal cells, which were covered with smooth cuticle. Cuticular sculptures may serve as excellent diagnostic characters, but their systematic significance for delimitation of categories below the species level is rather limited (Barthlott and Voit 1979). In all species examined in this study, SEM revealed the presence of epicuticular wax in the form of platelets, apart from *H. petiolaris*, whose achenes were without wax. Epicuticular secretions were well developed in *H. argophyllus* and *H. debilis*, but weakly developed in *H. annuus* and *H. praecox* (Figure 2F–J). Wax was distributed throughout the whole fruit surface, but the largest amount was located at the apical part of achene. Wax content can vary between locations

and environmental conditions, also temperature or water stress can enhance cuticular wax synthesis in several plant organs (Morrison 1984).

SEM analysis of mature achenes revealed the presence of multicellular, bi-seriate, non-glandular trichomes (twin hairs) (Figure 2E). Twin hairs are characteristic of Asteraceae and they consist of two elongated and parallel cells, originating from the anticlinal division of an epidermal mother cell (Cron *et al*., 1993). According to our results, in analyzed species hairs were equally distributed across the fruit surface, except for *H. argophyllus,* where trichomes were mainly distributed near the apical part of achene. Twin hairs were elongated and similar in length in all species. Karanović *et al*. (2016) also reported the presence of short, twin hairs in some other taxa of Asteraceae family. In all examined species, the nectaries were present at the apical part of achene. They were ring-shaped, surrounding the stylopodium base. The nectary epidermis was smooth without ornamentation and covered with cuticle. It contained numerous stomata with large pores between large guard cells (Figure 2C). Similar location and description of nectary glands of sunflower was reported by Sammataro *et al*. (1985).

CONCLUSION

The present study on mature fruit of wild annual sunflowers: *H. annuus, H. argophyllus, H. petiolaris., H. praecox,* and *H. debilis* showed that some examined characters such as dimensions of achene, carpopodium and stylopodium morphology, features of epidermal cells, presence of twin trichomes and nectaries morphology were rather similar in analyzed species. However, differences between the species were recorded in pigmentation of achene and development of epicuticular wax.

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МИКРОМОРФОЛОШКЕ КАРАКТЕРИСТИКЕ АХЕНИЈЕ ПОПУЛАЦИЈА ДИВЉИХ, ЈЕДНОГОДИШЊИХ ВРСТА СУНЦОКРЕТА

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РЕЗИМЕ: Циљ истраживања у овом раду је карактеризација једногодишњих врста сунцокрета на основу микроморфолошких параметара ахеније. Биљни материјал узгајан је на експерименталном пољу Института за ратарство и повртарство у Новом Саду. Узорци ахенија сакупљани су ручно током 2015. године, у време физиолошке зрелости. Морфолошка мерења извршена су применом стереоскопског микроскопа Leica M316 са Leica DFC 320 камером. Микроморфолошке карактеристике анализиране су помоћу скенинг електронског микроскопа (CEM). Добијени резултати указују на присуство квалитативних и квантитаивних разлика између анализираних врста, у параметрима као што су величина и боја ахеније, карактеристике карпоподијума и стилоподијума, као и дистрибуција трихома. Пронађена је разлика у орнаментици кутикуле и воска антиклиних зидова епидермалних ћелија на различитим деловима ахеније. CEM анализа указала је на присуство нежлезданих, вишећелијских, бисеријатних трихома (twin hairs). Наведени тип трихома састоји се од две издужене, паралелне ћелије различите дужине. Имајући у виду различиту расподелу трихома на апикалном, медијалном и базалном делу, већина врста се карактерише највећом густином трихома у апикалном делу ахеније.

КЉУЧНЕ РЕЧИ: ахенија, микроморфологија, трихоме