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DENSITY AND STRUCTURE OF LEAF TRICHOMES IN *Capsicum annuum* and *Capsicum frutescens*

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Abstract: Morphology and density of plant trichomes vary by species. Several studies on family Solanaceae have shown the diversity of trichomes among species and cultivated plants, but less research on density and morphology of trichomes in the genus *Capsicum*. The aim of this study is to determine the density and type of leaf trichomes from *Capsicum annuum* and *C. frutescens*. The structure of trichomes was observed under light microscope equipped with Dino Eye. The density of trichomes was recorded using stereo microscope as the number of trichomes per cm^2 multiplying the total area of the leaf. Glandular and non-glandular trichomes were covered on abaxial and adaxial surfaces of both species. No significant difference was observed for trichome density between adaxial and abaxial leaf surfaces within species (*C. annuum*, $t(8) = 1.37$, $p= 0.21$, and *C. frutescens*, $t(8) = -0.23$, $p= 0.82$). However, mean trichome density on the abaxial surfacesof *C. annuum* was significantly lower than *C. frutescens* ($F (3,16) = 3.79$, $p= 0.03$). The density of glandular trichomes occurred on leaf midrib of *C. frutescens* (8.17 ± 1.22 cm^{-2}) was higher than *C. annuum* (4.20 ± 0.42 cm^{-2}; $t(8) = -3.74$, $p= 0.02$). This study provides basic knowledge of leaf trichomes structure and the density of cultivated chili plants in Malaysia.

Keywords: Morphology, Solanaceae, chili pepper.

Introduction

Plant trichomes are epidermal growth structure that are present on surfaces of leaves, stems, petals, petioles and peduncles. Trichomes serve as effective defenses against herbivores by preventing the feeding and oviposition of phytophagous arthropods, or secreting toxic chemicals to entrap, deter and poison them (Champagne & Boutry, 2016, Kariyat *et al.*, 2017, Chen *et al.*, 2018). Trichomes also protect plants from wind and UV radiation (Bickford, 2016).

The morphology and density of trichomes vary greatly among species and are used to distinguish between closely related species and hybrids (Dalin *et al.*, 2008). Trichome structures can range from unicellular to multicellular, with glandular or non-glandular, straight, spiral, branched or non-branched (Talip, 2019). In most of the species, individual plants can produce more than one type of trichome. For instance, non-glandular trichomes act as mechanical barrier for arthropods (Kariyat *et al.*, 2018), whereas glandular trichomes provide physiochemical defense against pathogens and pests by producing toxic substance like terpenoids, phenolics and alkaloids (Murungi *et al.*, 2016, Chen *et al.*, 2018).

Trichomes on Solanaceae are reported playing roles as primary defense against pest (Tingey, 1991, Wilkens *et al.*, 1996, Tian *et al.*, 2012, Kariyat *et al.*, 2017, Chen *et al.*, 2018). For instance, glandular trichomes on tomato leaves produce terpenes volatiles to attract the predator of thrips (Escobar-Bravo *et al.*, 2017, Chen *et al.*, 2018). However, trichome studies in cultivated chili, *Capsicum* sp. in Malaysia, are still limited. Adedeji *et al*. (2007) and Kim *et al*. (2012) have described trichome structures in *C. annuum*, *C. frutescens* and *C. chinense,* but their studies do not discuss the level of defense in plants. Hence, the aim of this study is to determine the density and structure of leaf trichomes in *C. annuum* and *C. frutescens*. Different number of trichomes based on position and structure may explain the defense mechanism in the species.

Materials and Methods

Plant Material

The *Capsicum frutescen*s fruits (variety PELITA 8) were purchased at a supermarket and *Capsicum annuum* seeds (variety SAKATA 461) were bought from GM Peladang, Kuala Terengganu. The varieties are the most cultivated chili crops in Malaysia. The fruits of *C. frutescens* were dried in a dryer for 24 hours at 40. Seeds from both species were soaked in water overnight and dried at room temperature for eight hours to prevent fungi infection during storage. The seeds were sown in peat soil (FreePeat, Netherlands) the next day. The plants were placed in an insect proof cover without any pesticide. Plants which were two months old were used for further experiments.

Morphology of Trichomes

Young and expanded leaves were collected from nodes eleventh and twelfth of a plant. The morphology of trichome was observed under light microscope, equipped with Dino eye AM4023 (AnMo Electronics Corporation). The morphology of trichomes was further classified according to Talip *et al*. (2019).

Density of Trichomes

The density of trichomes was observed using stereomicroscope. Under stereomicroscope, 0.25 cm X 0.25 cm grid of overhead projector (OHP) paper were placed above the leaf. The number of trichomes was calculated on five replicate plants per species and each replicate represented an average of trichomes number from eleventh and twelve nodes of leaflet. The total number of trichomes on adaxial, abaxial and midrib of leaf surface was recorded. The density of trichomes was calculated by dividing the total cell number of trichomes by leaf area and were expressed in cm^{-2}.

Data Analysis

The data analysis was conducted using SPSS ver. 22. Comparison of mean trichome density between *C. frutescens* and *C. annuum* were analyzed using One-Way ANOVA. Comparison of mean density of glandular trichomes at midrib was analyzed using Independent T-Test.

Results and Discussion

Both *Capsicum annuum* and *C. frutescens* possessed non-glandular and glandular trichomes. Non-glandular trichomes on leaf surfaces have simple structures with multicellular stems, unicellular base and conical tips (Fig. 1A and 1C). One type of glandular trichome was observed on leaf epidermis of both species. This type of trichome has a short one cell stem, and multicellular gland cells on the tip of trichomes (Fig. 1B and 1D).

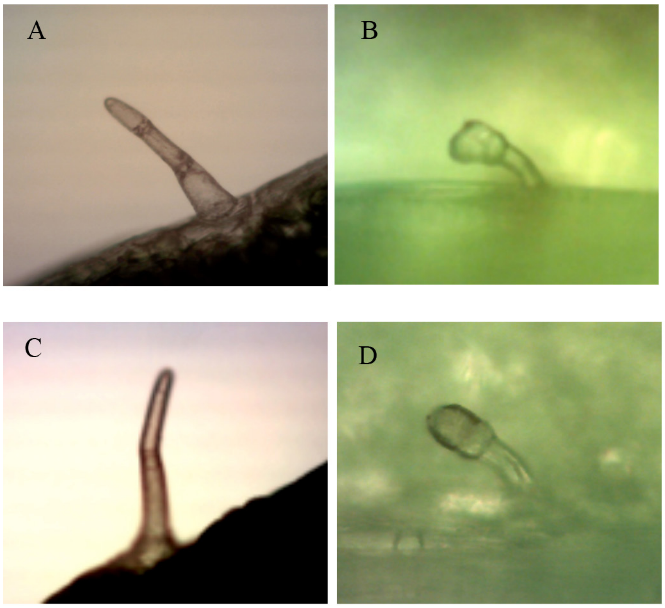

Figure 1: Morphology of trichomes on the leaf surface of *C. annuum* (A, B) and *C. frutescens* (C, D) at 700x magnification

In this study, leaf of *C. annuum* var. annuum possessed both non-glandular trichomes and glandular trichomes, which were similar with the finding by Kim et al. (2012). Multicellular stem non-glandular trichomes and glandular capitate trichomes were present on both abaxial and adaxial surfaces (Kim *et al.*, 2012). In contrast, Abediji *et al.* (2007) reported that non-glandular trichomes were absent on leaf surface of *C. annuum*. While Malaysian cultivated *C. frutescens* leaves have both glandular and non-glandular trichomes, Kim *et al.* (2012) reported that only glandular trichomes occurred on leaf surface of their *C. frutescens*. The differences can be associated to the influence of parent cultivars, photoperiod, type of fertilizers and soils.

The mean density of trichomes on leaf epidermis of *C. annuum* and *C. frutescens* was 6.4 $\pm$ 2.7 cm^{-2} and 15.2 $\pm$ 2.6 cm^{-2}, respectively. No significant difference was observed for trichome density between adaxial and abaxial leaf surfaces of *C. annuum* ($t(8) = 1.37$, $p=0.21$) and *C. frutescens* ($t(8) = -0.23$, $p=0.82$). Trichome density ~~on~~ was significantly higher on abaxial surface of *C. frutescens* (7.83 $\pm$ 1.90 cm^{-2}) compared with *C. annuum* (1.83 $\pm$ 0.96 cm^{-2}; $F(3,16) = 3.79$, $p=0.03$) (Fig 2). In both species, glandular trichomes were mostly distributed along the midrib of leaf surface. The density of glandular trichomes present on midrib was significantly higher in *C. frutescens* (8.17 $\pm$ 1.22 cm^{-2}) compared to *C. annuum* (4.20 $\pm$ 0.42 cm^{-2}; $t(8) = -3.74$, $p= 0.02$) (Fig 3).

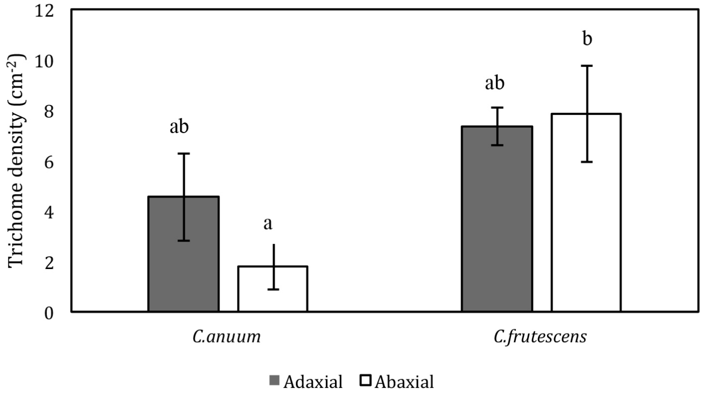

Figure 2: Mean density of leaf trichomes from *C. annuum* and *C. frutescens*. Data show the mean (± SE) trichome number of five replicates. Different letters indicate significant differences among treatments (One-way ANOVA followed by Tukey's post hoc analysis: $p < 0.05$)

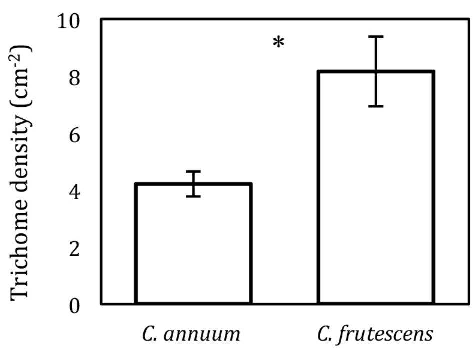

Figure 3: Density of glandular trichomes at midrib of *C. annuum* and *C. frutescens*. Data show the mean (± SE) trichome number of five replicates. Asterisks represent significant difference of trichome density between species (T-Test: $p<0.05$)

In general, the number of trichomes per cm^2 on *C. frutescens* leaf surfaces was higher than *C. annuum*, suggesting that *C. frutescens* have better defense strategy than *C. annuum*. Low number of trichomes on abaxial indicated that this area ~~is~~ was susceptible to the phytophagus attack. Low density of trichomes on abaxial promoted the high infestation of herbivores on tomato plant (Campos *et al.*, 2009, de Oliveira *et al.*, 2018). Trichomes have been shown to be directly and indirectly involved in defense of chili plants. For instance, thrips (*Scirtothrips dorsalis*) avoided high number of trichomes that limited the movement of the chili thrips (Latha & Hunumanthraya, 2018). *Capsicum annuum* also emitted terpene volatiles that deter whitefly (*Bemisia tabaci*) from settling on *C. annuum* (Saad *et al.*, 2019). Terpene volatiles have been reported to emit from glandular trichomes of tomato, which reduce the thrips infestation on plants (Escobar-Bravo *et al.*, 2017, Chen *et al.*, 2018). Glandular trichomes also secrete protein inhibitor, phylloplanins from *Solanum tabacum,* which inhibits the spore germination and infection of pathogen *Peronospora tabacina* (Shepherd *et al.*, 2005). The data from this study, nevertheless can only explain the constitutive defense of chili plants, although the number of trichomes may change upon pest infestations in *C. frutescens* and *C. annuum*.

Apart from their defensive role against biotic stress, less research ~~is~~ has established ~~for~~ their function in abiotic stress. High density of non-glandular trichomes prevents the absorption of UV-B into plant tissues (Holmes & Keiller, 2002, Tattini *et al.*, 2007, Yan *et al.*, 2012). Dense non-glandular trichomes also control the diffusion of water vapour during transpiration in order to reduce the water losses and regulate the energy balance and temperature of the lamina (Konrad *et al.*, 2015, Pshenichnikova *et al.*, 2018). Thus, the chili trichomes may also serve to protect the plants from abiotic stress as well, and biochemical and physiological studies are required to elucidate the role of trichomes in chili plants.

Conclusion

The trichome density in chili leaves between *C. annuum* and *C. frutescens* is different, suggesting that *C. frutescens* is likely to provide better protection against biotic and abiotic stress than *C. annuum*. The different roles of non-glandular and glandular trichomes can be the subjects of future investigations.

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