Scholarly Journal of Agricultural Science Vol. 8(1), pp. 1-6 March, 2018
Available online at http:// www.scholarly-journals.com/SJAS
ISSN 2276-7118 © 2018 Scholarly-Journals

*Full Length Research Paper*

# Comparision of the leaf Stomatal characteristics between *Brassica campestris* and *B. napus* (Brassicaceae: *Brassica*)

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Accepted 10 March, 2018

**Stomata are consequential as gaseous and dihydrogen monoxide exchange organs for most of the plants and are proximately cognate to photosynthesis, respiration and transpiration. In this study, we compared the stomatal characteristics of epidermis of leaves of Brassica campestris and B. napus, two economically consequential plant species in Brassica (Brassicaceae). Leaves of both species are dorsoventral. Types of stomata observe on upper surface of B. campestris leaf were diacytic, anisocytic, holoparacytic, hemiparacytic and anomocytic and on lower epidermis were hemiparacytic, anomocytic and anisocytic stomata. Stomatal types observe on upper epidermis of B. napus were anomocytic, desmocytic, hemiparacytic and anisocytic and on lower epidermis of B. napus were anomocytic anisocytic and diacytic. In B. campestris the highest stomatal density was recorded on the adaxail side while in case of B. napus it was highest on the abaxial surface. Percentage of closed stomata was more on the abaxial side of both species as compared to the adaxial side. There were diminutive distinctions between mean length and width of the stomatal pores and sentinel cells of the adaxial and abaxial surfaces of the both plant species. There was a positive paramount cognation between the epidermis and stomata of the same leaf surface in both plant species. It is concluded from the present study stomata on the leaves surfaces are bio-designators. Their numbers, arrangement, types, distribution, indices, densities and frequencies pellucidly show the type of the environment in which the study plants grow.**

**Key words:** BN = Brassica napus, BC = Brassica campestris, L = Lower epidermis, U = Upper epidermis, U = Upper epidermis

## INTRODUCTION

The utilization of epidermal characters such as stomata types, stomatal frequency and index seem to be incrementing rapidly because not only do epidermal characters correlate with gross morphological features in most cases, they are often kenned to be very valuable at the caliber where the classical methods of cytological and genetics cannot be applied (Stace 1965). Many workers showed that leaves possess many morphological characters of potential taxonomic paramountcy that are often diagnostic at the genus and species level (Arroyo, 1985, Aina, 2013). The shape of the epidermal cells, types and arrangement of stomata, size and shape of trichomes are consequential systematic parameters (Mbagwu, 2005). Stomata sanctioned the higher plants to acclimate to virtually all terrestrial environments on the planet, by betokens of adjustment of their size, density and distribution (Zarinkamar, 2006). Stomata are paramount as gaseous exchange organs for most of the plants. They occur either on one or on both surfaces of leaf (Parveen et al., 2007), Dilcher. (1974), (Stebbins and Khush 1961), (Stace, 1969, 1973) reported that stomata are taxonomically paramount and can be utilized as an

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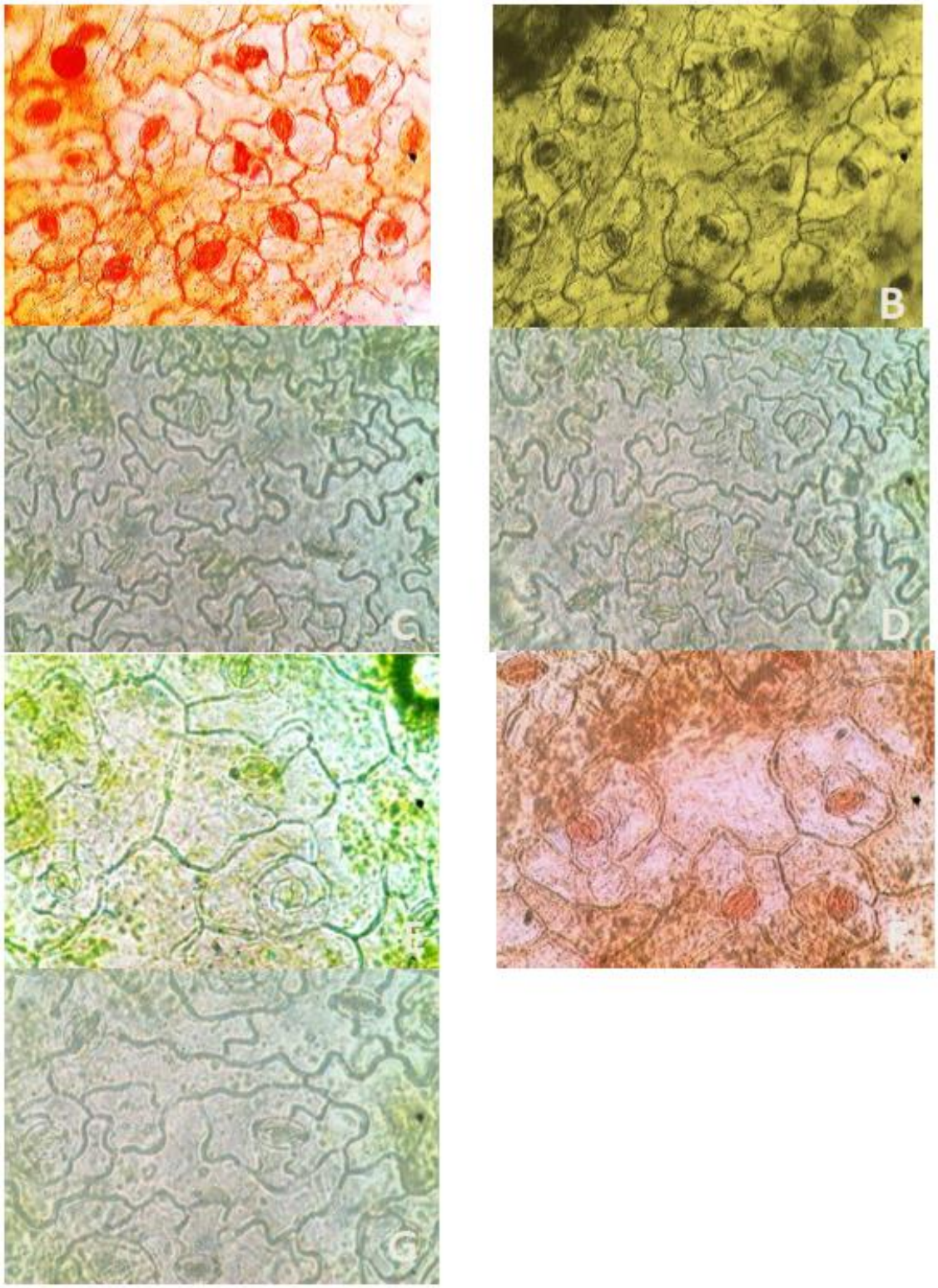


**Figure 1** Stomata types of *B*. *campestris* (**A**: anomocytic, anisocytic and hemiparacytic stomata on dorsal side; **B**: irregular polar, anomocytic and diacytic stomata on dorsal side; **C**: anomocytic stomata on ventral side; **D**: anomocytic, hemiparacytic and anisocytic stomata on ventral side) and *B*. *napus* (**E**: anomocytic and anisocytic stomata on dorsal side; **F**: anisocytic and diacytic stomata on dorsal side; **G**: Desmocytic, anomocytic, hemiparacytic and anisocytic stomata on ventral side).

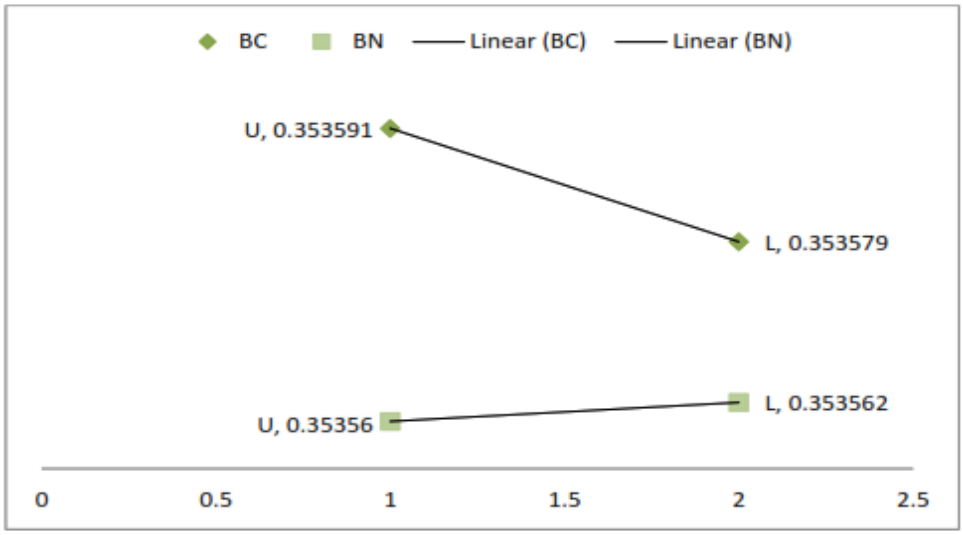


**Figure 2.** Pearson product moment coefficient showing linear relation between stomata and epidermis within the same leaf surface

efficient implement for higher caliber of taxonomic hierarchy. According to (Srivastava and Singh, 1972) the stomata are minute apertures circumvented by 2 sentinel cells (kidney shaped) controlling opening and closing of stomata. Sundry scientists approved that there are 31 distinct types of stomata and they utilize these variants of stomata as a taxonomic implement in relegation of plants at higher caliber hierarchy (Dilche, 1974, Fryns-Claessens and Cotthem 1973). An alteration of leaf stomatal density can be utilized as a be speaker of environmental change (Case, 2004). Several researchers have shown that stomatal densities vicissitude in replication to dihydrogen monoxide availability (Edwards and Meidner 1978) light intensity [Retallk, 2001, Lu et al., 1993), temperature (Ciha and Brun, 1975), geographical location [Retallk, 2001).and CO2 concentration (Bristow and Looi, 1968, Woodward and Bazzas, 1988).

Some of choline ester present in different components of different species of genus Brassica such as isoferuloylcholine, hepsperaline, sinapine and 4-Hydroxybenzolcholine are utilized as implement for genus characteristic in Brassicaceae. Their distribution both qualitatively and quantitatively utilized in chemotaxonomy. The accumulation of phenolic ester is variable in different species of genus Brassica seeds (Bouchereau et al., 2001).

Run-hui et al., [2002] study micro-morphological of leaf epidermis in 26 species of Bamboo ([Keating, 2002) withal study anatomical characteristic of leafs in some species of Araceae for phylogenetic analysis. Little work and research on this task has been done in Pakistan [Zahur and Parveen 1982, Chaudhary and Imran, (1997). Detailed anatomical study of leaf epidermis and stem of Brassica compestris and Brassica napus has not been studied yet. In this article, investigation has been made to ascertain variants of stomata, length and width of stomata and sentinel cell, and statistical analysis of stomata and epidermal cell of two species of Brassica.

## MATERIALS AND METHOD

Brassica compesris and Brassica napus were accumulated from Peshawar, Pakistan. The geolocation of Peshawar is on the north and east hemisphere. Its area is 460 sq mi 1,257 km2. It lies at 34˚ 0' 15" N, 71˚ 32' 41" E. Plants were identified by Prof. Dr. Abdur Rashid, Taxonomist, Department of Botany University of Peshawar, Pakistan by following Flora of Pakistan family Brassicaceae (Jafri, 1973). Very puerile leaves of the plants were culled. For microscopy concentration was made on middle portion of the plants. The leaf epidermis of both plants was utilized for observation of stomatal types and their different sizes of sentinel cells, and epidermal cells at 40 magnification puissance. 10 readings were taken during the present investigation. Peels from both surfaces (adaxial and abaxial) were obtained through razor.

The obtained peels were fine-tuned on slides with the avail of canada balsam and pass on through microscopic examination for stomatal study (Chaudhary and Imran, (1997). The photographs were taken by Nikon FDX-35 Microscope. Sizes of stomata, sentinel cells and epidermises were quantified by ocular micrometer of both adaxial and abaxial surfaces of leaf epidermis following a method of [Singh, 2009]. Calculation of Stomatal index was carried out by a formula given by Meinder and Mansfeid [1968]. Statistical evaluation was carried out to ascertain the correlation between the stomata and epidermis of the same surface of the leaf of the plant by

**Table 1.** Types of stomata, Stomatal index, Density, Frequency, percentage of close and open stomata in *Brassica campestris* and *Brassica napus* of Brassicacea.

| Species | Types of stomata | Density of stomata | Frequency of stomata | % age of open stomata | % age of close Stomata | Stomatal index |
|---|---|---|---|---|---|---|
| *Brassica campestris* (upper) | Anomocytic | 24.76 | 2476 | 44.11 | 55.88 | 46.9 |
| | Anisocytic | 40.1 | 4010 | | | |
| | Hemiparacytic | 36.19 | 3619 | | | |
| | Diacytic | 15.23 | 1523 | | | |
| | Holoparacytic | 11.42 | 1142 | | | |
| *Brassica napus* (upper) | Anisocytic | 22.85 | 2285 | 54.76 | 41.66 | 41.66 |
| | Anomocytic | 24.76 | 2476 | | | |
| | Hemiparacytic | 15.2 | 1520 | | | |
| | Desmocytic | 17.14 | 1714 | | | |
| *Brassica campestris* (lower) | Anomocytic | 41.90 | 4190 | 61.64 | 38.35 | 47.09 |
| | Hemiparacytic | 40.21 | 4021 | | | |
| | Anisocytic | 43.80 | 4380 | | | |
| *Brassica napus* (lower) | Anomocytic | 22.85 | 2285 | 72.72 | 27.27 | 30.74 |
| | Diacytic | 20.95 | 2095 | | | |
| | Anisocytic | 19.04 | 1904 | | | |

Pearson's product moment coefficient following Choudhary and Kamal [2004]. Stomatal index of both surfaces were found by utilizing following formula:

Stomatal index = Number of stomata/unit area×100

Number of stomata /unit area + Number of epidermal cells
The relationship between the epidermis and stomata within the same leaf was resolute by the formula

$$r_{xy} = \frac{\sum(XY - nX_1Y_1)}{nS_XS_Y}$$

where

r is the Pearson product moment coefficient with deference to stomata and epidermis;
X is the number of stomata; Y is the number of epidermal cells;
X1 is the cumulative mean value for stomata;
Y1 is the cumulative mean value for epidermal cells;
SX is the standard deviation for the stomata;
SY is the standard deviation for epidermis; n is the number of slides.

To ascertain the paramount cy/insignificance between the epidermis and stomata of the same leaf surface the following equation was utilized following

$$t = \frac{R}{\text{sqrt}[(1—r^2)/(N—2)]}$$

## RESULTS

Types of stomata on upper surface were anomocytic, anisocytic, hemiparacytic, diacytic and holoparacytic and on lower surface were anomocytic, anisocytic, hemiparacytic of B. campestris. While in B. napus stomata on upper surface were anomocytic, anisocytic, hemiparacytic and desmocytic and on lower surface were anomocytic, diacytic and anisocytic. The highest density was 40.1 for anisocytic and lowest 11.42 for holoparacytic on the adaxial surface of B. campestris, while on the abaxial surface highest 43.80 for anisocytic and lowest 40.21 per mm2 for hemiparacytic stomata. In case of B. napus the anomotetracytic stomata had the highest 24.76 density and hemiparacytic had the lowest 15.2 density on the adaxial side while on the abaxial surface highest 22.85 for anomotetracytic and lowest 19.04 per mm2 for anisocytic stomata. In both plant species the percentage of the opened stomata was more on the abaxial surface as compared to the adaxial surface. In B. compestris it was 61.64 while in B. napus it was 72.72. Percentage of closed stomata was more on the abaxial side of both species as compared to the adaxial side. In B. campestris adaxial surface had more (55.88) close stomata as compared to the abaxial surface (38.35). Similarly in B. napus the adaxial surface had more (41.66) stomata comparing to the adaxial surface (27.27). In both species stomatal index was high on the abaxial side as compared to the adaxial side. It was 47.09 on the abaxial surface and 46.9 on the adaxial side of the B. campestris while in case of B. napus it was 41.66 on the abaxial side and 30.74 on the adaxial side of the plant species (Table 1). The mean length of sentinel cell ranged from 21.3mm (abaxial surface) – 23.5mm (adaxial surface) of B. campestris and 23.5mm (adaxial side) – 25.5mm (abaxial side) of B. napus.Types of stomata on upper surface were anomocytic, anisocytic, hemiparacytic, diacytic and holoparacytic and on lower surface were anomocytic, anisocytic, hemiparacytic of B. campestris. While in B. napus stomata on upper surface

**Table 2.** Average size of guard cells and stomatal pore in both surfaces of leaf in *Brassica campestris* and *Brassica napus* of Brassicaceae.

| Species | Mean size of guard cell | | Mean size of stomatal pore | |
|---|---|---|---|---|
| | Length | Width | Length | Width |
| *B. campestris* upper | 23.5 | 5.5 | 20.4 | 2.8 |
| *B. campestris* lower | 21.3 | 5.53 | 18.25 | 1.98 |
| *B. napus* upper | 23.5 | 7.58 | 21.5 | 2.1 |
| *B. napus* lower | 25.5 | 6.25 | 22.5 | 1.80 |

were anomocytic, anisocytic, hemiparacytic and desmocytic and on lower surface were anomocytic, diacytic and anisocytic.

The highest density was 40.1 for anisocytic and lowest 11.42 for holoparacytic on the adaxial surface of B. campestris, while on the abaxial surface highest 43.80 for anisocytic and lowest 40.21 per mm2 for hemiparacytic stomata. In case of B. napus the anomotetracytic stomata had the highest 24.76 density and hemiparacytic had the lowest 15.2 density on the adaxial side while on the abaxial surface highest 22.85 for anomotetracytic and lowest 19.04 per mm2 for anisocytic stomata. In both plant species the percentage of the opened stomata was more on the abaxial surface as compared to the adaxial surface. In B. compestris it was 61.64 while in B. napus it was 72.72. Percentage of closed stomata was more on the abaxial side of both species as compared to the adaxial side. In B. campestris adaxial surface had more (55.88) close stomata as compared to the abaxial surface (38.35). Similarly in B. napus the adaxial surface had more (41.66) stomata comparing to the adaxial surface (27.27). In both species stomatal index was high on the abaxial side as compared to the adaxial side. It was 47.09 on the abaxial surface and 46.9 on the adaxial side of the B. campestris while in case of B. napus it was 41.66 on the abaxial side and 30.74 on the adaxial side of the plant species (Table 1). The mean length of sentinel cell ranged from 21.3mm (abaxial surface) – 23.5mm (adaxial surface) of B. campestris and 23.5mm (adaxial side) – 25.5mm (abaxial side) of B. napus.

## DISCUSSION

Stomatal study of Brassica campestris and Brassica napus is carried out. Different parameter such as types of stomata, stomatal index, average size of sentinel cell, average size of stoma, average of opened and closed percent of stomata on each surface of leaf. Statistical analysis was withal carried out. Results obtained after detailed study are further utilized in taxonomic study of plants at molecular level.

Stomatal function is consequential on the physiology, adaptation and productivity of the plants. The number and distribution of stomata per unit leaf area have consequential roles in these processes by adjusting CO2, O2 and moisture exchange between the leaves and atmosphere (Brownlee 2001). There are immensely colossal variation in the number and structure of stomata in per unit area among plant species and leaves [Peksen et al., 2014).

Variants of stomata have been reported by different workers in different plants species like in *Melilotus indicus, Datura alba* and *Tribulus terrestris* have anomocytic stomata while *Calotropis procera* and *C. brachycarpa* have paracytic type of stomata (Parveen et al., 2007), anisocytic, paracytic, tetracytic, hexacytic, hemiparacytic in different plants belonging to the family Polygonaceae (Hameed et al., 2008), anisocytic, paracytic, amphianisocytic, anomotetracytic and in some members of family Solanaceae (Hameed and Hussain, 2011), staurocytic, amphianisocytic and paracytic stomata in 36 plants accumulated from district Tank (KP, Pakistan) (Ahmad et al., 2009).anisocytic stomata had been reported in the family Brassicaceae (Metcalfe and Chalk (1979, Berger and Altmann 2000). Anisocytic stomata additionally reported in *Alyssum floribundum* (Orcan and Binzet, (2004), *Alyssum desertorum, A. minutum, Nelsia apiculate,* and *Turritis glabra*, anisocytic and anomocytic (*Alyssum sibiricum, Alyssum strigosum, Alliaria petiolate, Hesperis hyrcana*) (Zarinkamar 2007) which accede with the present study.

In the present study stomatal frequency for anisocytic and anomocytic in B. campestris was lower in adaxial surface as compared to the abaxial surface while for anomocytic, anisocytic and hemiparacytic in B. napus was higher for the adaxial surface as compared to the abaxial surface. Our results is fortified by Cole and Dobrenz, (1970), in alfalfa, Shearman and Beard (1972) in creeping grass, Tann and Dunn (1975) on the flag leaf of wheat for *B. napus* but in contradiction with *B. campestris*. According to Willmer and Fricker (1996), stomatal frequency and sentinel cell size are interdependent and the stomatal size conventionally decreases with incrementing stomatal density.

However, the data presented in this paper shows that sentinel cells on lower leaf surfaces are more diminutive in size in *B. napus* than those of the upper surfaces in *B. campestris*. Table 2 show that Willmer and Fricker (1996) observations generally applied to the woody plants, however, it can additionally be applied to herbaceous plants with some exceptions such as *B. campestris* and *B. napus* (Brassicaceae). In these exceptions, in spite of low stomatal densities on the adaxial surfaces, the

abaxial sentinel cells are more immensely colossal than those of the adaxial surface.

So it is concluded from the present study stomata on the leaves surfaces are bio-designators. Their numbers, arrangement, types, distribution, indices, densities and frequencies limpidly show the type of the environment in which the study plant/s grow.

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