



Micromorphological, Anatomical and Biochemical Analysis of some Divisive *Capsicum Annuum* Varieties of North East India

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Received: 13 July 2020 / Revised: 1 September 2022 / Accepted: 2 September 2022
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Abstract The varieties of *Capsicum annuum* L. available in north eastern part of Assam looks strikingly indistinguishable and researchers often struggle to differentiate between them in their investigations. The present experiment was designed to investigate the variation among the eight varieties of *C. annuum* regarding their morphological, anatomical and biochemical features. The results reveals significant variations in morphological characters such as size of the flower, the colour of corolla, length of petiole, length of leaf, length of fruit and number of seeds; anatomical variation was prominent in vascular bundle arrangement while Capsaicin content provides meaningful evidence regarding their biochemical variations.

Keywords Chillies · Bhoot jolokia · Capsaicin · Capsicum

Introduction

Chilli is native to South and Central America and consists of annual or perennial herbs or shrubs. The genus *Capsicum* L. belongs to one of the largest plant family Solanaceae, which include crops with economic importance like potato and tomato [1]. The subfamily and tribe of Capsicum is Solanoideae and Capsiceae, respectively. Chillies are usually self-pollinated crop but they can opt for cross-pollination and the probability of a self-pollinated chilly plant to turn into cross pollinated ranges from 7% to 90% [2]. The members of the genus Capsicum has the capability to interbreed successfully based on which they can be grouped into categories and the rate of inbreeding was found to be very high making it complex to categorize and it can create some undistinguishable characters among the varieties [3]. The genus Capsicum is composed of around 25 species in the wild and five species (*Capsicum chinense* Jacq., *Capsicum frutescens* L., *Capsicum annuum* L., *Capsicum baccatum* L., *Capsicum pubescens* Ruiz & Pav) are domesticated [4]. Among the states of North East India, Assam produces the largest quantity of chilli with a production of 10,000 tonnes in 16,000 hactor area. Naga chilli, commonly known as “Bhoot jolokia” is renowned to the world scientific community and regarded as the hottest chilli of the world [5]. A huge area for cultivation of chilli is utilized in most of the parts of North Eastern regions of India most of which are local cultivars or landraces adapted to the local climatic conditions and undergoes selection and maintenance by the farmers for their ecological, economic, social and cultural needs. *C. annuum* is spread throughout the globe with phenotypic diversity such as in colours, shapes, sizes of the fruits. Morphological traits in chillies occur in great variation in all over India like in the southern peninsular region, North Eastern foothills of the Himalayas and Gangetic plains [6].

Significance statement: The distinct morphological, anatomical and biochemical variations among the eight varieties of *C. annuum* L. were seem to vary considerably. There is a decent scope for future researchers to upgrade some of these varieties to new ‘species’ level.

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After an extensive literature survey, the authors realised that a lot of emphasis has been given on separation of varieties from one another in the molecular level although very few research works were based on their morphological, anatomical and biochemical specifications which can easily mislead the upcoming breed of researchers in many way. There is always a chance of choosing a wrong variety of the species for further processing regarding molecular and germplasm characterization. A very sound knowledge about the morphological and anatomical characters of the plant species can provide enough information for its separation from other related species of the same genus without even going into the molecular details [7]. Too much reliance on molecular taxonomy is causing significant problems in addressing the basic taxonomic issues and most of the time many important confusions remains unnoticed for years. This research paper is exclusively based on clearing the doubts utilizing basic taxonomic characterization of 8 *C. annuum* varieties followed by biochemical confirmation to put forward a clear picture of the species for designing future research idea in this field.

Material and Methods

Collection of the Plant Materials

Fresh plant materials were collected from various farms and households of various parts of North East India. From the field live plants were collected to perform the experiments and for preparation of herbarium. The methodology suggested by Jain and Rao (1977) [8] was followed for pressing and preparation of herbarium specimens.

Identification and Nomenclature

Extensive literature survey [9, 10] was carried out to identify the specimens at the preliminary level and was later confirmed by consulting ASSAM herbarium of Botanical Survey of India, Shillong.

Morphological and Anatomical Characterization

A complete and detailed morphological investigation of the specimens was carried out. For anatomical characterization, the standardized anatomical procedures were followed for dissection along with two different stains namely 4% aqueous safranin and toluidine blue.

Biochemical Characterization

Capsicum fruits were dried and coarsely powdered using a mechanical grinder; 10 g of this powder is subjected to maceration with acetone for 24 h. The acetone extracts was

collected after filtration. Spectrophotometric method proposed by Sasasivam and Manikam (1992) [11] was utilized for estimation of capsaicinoids content.

Stomatal Index

Stomatal index is defined as the percentage which the numbers of stomata appeared to the total number of epidermal cells, each stoma being considered as a single cell. Stomatal index can be calculated by using following equation [12].

$$SI = S \times 100 / E + S$$

SI = Stomatal index, S = No. of stomata per unit area, E = No. of epidermal cells in the same unit area.

Results and Discussion

Morphological Variation

Morphological characters among 8 varieties of *C. annuum* L. is presented in Table 1 and photographs were presented in Figs. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10. It gives a fair amount of idea about the morphological difference between the eight chosen varieties of *C. annuum* which is easily visible to the naked eye and can be act as an indicator for differentiation of the varieties from one another. The flower size ranges from 0.8 to 1.2 cm; the colour of corolla ranges from purple, white and off-white; length of petiole ranges from 0.7 to 3 cm; length of leaf varies from 4 to 6.5 cm across the varieties; fruit length was found in the range of 1.8–8 cm and number of seed varies from 11 to 80. Striking similarities between calyx and gynoecium was recorded which is one of the many factors that are responsible for misleading the researchers regarding the proper identification of the varieties. The calyx remain persistent and 5-toothed irrespective of the varieties while gynoecium was found with capitate stigma in all the varieties.

Anatomical Variation

Anatomical characters among the varieties of *C. annuum* L. is presented in the Table 2 and photographs were provided in Fig. 11, 12, 13, 14, 15. Transverse section of the petiole reveals epidermal hair in six of the eight varieties. *C. annuum* var. *fasiculatum* and *C. annuum* var. 'goat horn' was the only two varieties which did not produce any epidermal hair. Differences in the arrangement in vascular bundle of the petioles of eight varieties were noticed in the form of fragmented, non-fragmented and c-shaped. T.S of stem confirmed absence of epidermal hair in *C. annuum* var. *fasiculatum*, *C. annuum* 'eagle claw' and *C. annuum* var.

Table 1 Morphological characters among varieties of *Capsicum annuum* L

Varieties	Flower size (cm)	Calyx	Corolla with numbers of stamen	Gynoecium	Petiole length (cm)	Leaf length (cm)	Fruit length with number of seeds
<i>C. annuum</i> var. longum	1–1.2	Persistent, 5-toothed	Purple; Stamen 5	Capitate stigma	2.5–3	6–6.5	3.5–4 cm; 11–20
<i>C. annuum</i> var. fasciculatum	1–1.2	Persistent, 5-toothed	White; Stamen 5	Capitate stigma	1.5–2	5–5.5	4–4.5 cm; 20–30
<i>C. annuum</i> ‘eagle claw’	0.8–1	Persistent, 5-toothed	Off-white; Stamen 5	Capitate stigma	1–1.7	5.5–6	5–8 cm; 48–55
<i>C. annuum</i> var. annuum	0.6–0.8	Persistent, 5-toothed	Off-white; Stamen 5	Capitate stigma	0.7–1.3	5.8–6.2	3.5–4 cm; 49–55
<i>C. annuum</i> var. ‘goat horn’	8.8–0.9	Persistent, 5-toothed	White; Stamen 5	Capitate stigma	1.2–1.8	4–4.5	6–8 cm; 25–30
<i>C. annuum</i> var. ‘Korean long green’	0.9–1.1	Persistent, 5-toothed	White; Stamen 5	Capitate stigma	1.2–1.8	4.8–5.3	5.3–6 cm; 72–80
<i>C. annuum</i> var. conoides	0.9–1	Persistent, 5-toothed	White; Stamen 5	Capitate stigma	1.8–2.8	4.5–5	5.4–6 cm; 48–55
<i>C. annuum</i> var. ‘cascabel’	0.9–1.2	Persistent, 5-toothed	White; Stamen 5	Capitate stigma	0.9–1.4	4–4.9	1.8–2 cm; 18–22

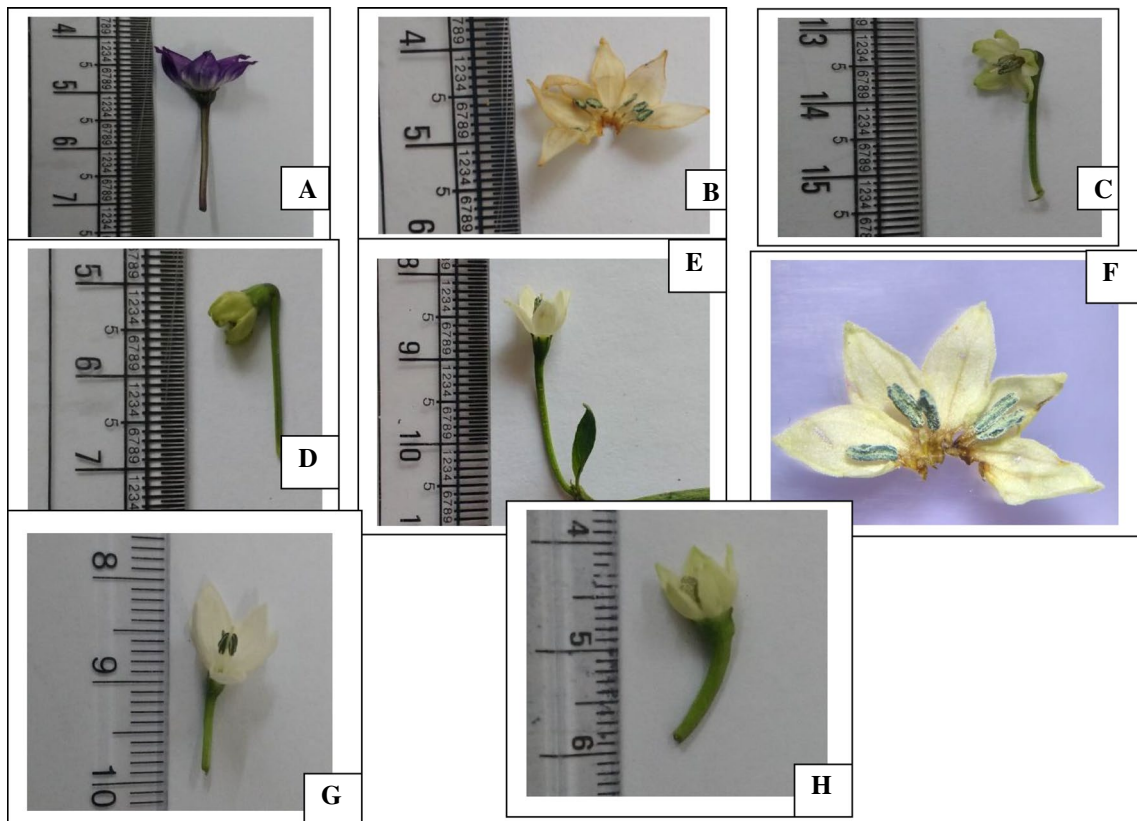


Fig. 1 Complete flower of the eight varieties of *Capsicum annuum* L. **a**—*C. annuum* var. longum; **b**—*C. annuum* var. fasciculatum; **c**—*C. annuum* ‘eagle claw’; **d**—*C. annuum* var. annuum; **e**—*C. annuum*

‘goat horn’; **f**—*C. annuum* ‘Korean long green’; **g**—*C. annuum* var. conoides; **h**—*C. annuum* ‘cascabel’

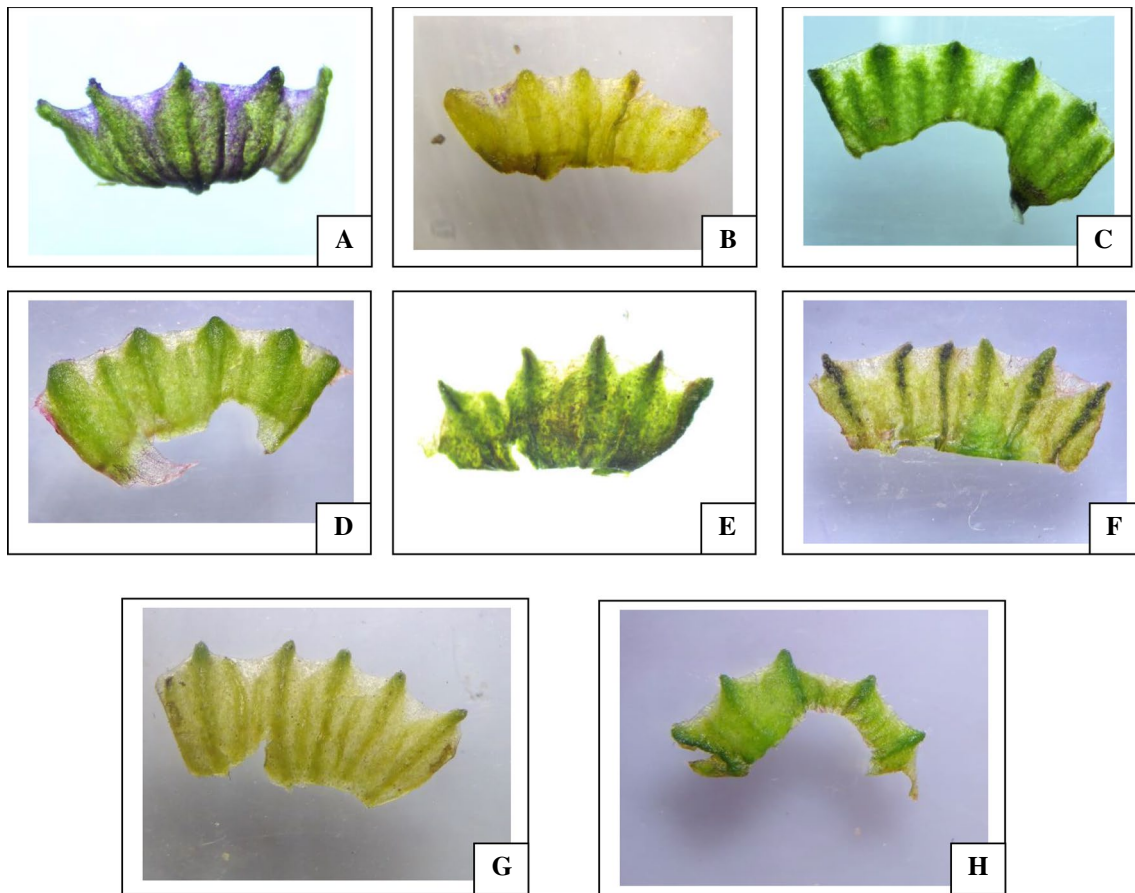


Fig. 2 Calyx of the eight varieties of *Capsicum annuum* L. **a**—*C. annuum* var. longum; **b**—*C. annuum* var. fasciculatum; **c**—*C. annuum* 'eagle claw'; **d**—*C. annuum* var. annuum; **e**—*C. annuum* 'goat horn';

f—*C. annuum* 'Korean long green'; **g**—*C. annuum* var. conoides; **h**—*C. annuum* 'cascabel'

annuum. Differences in the layers of cortex ring were also recorded among the varieties. Stomatal Index (SI) of adaxial surface of leaf ranges from 3 to 17% and even in one variety, *C. annuum* 'cascabel' it was found that the SI was zero; Stomatal Index (SI) of abaxial surface of leaf ranges from 17 to 29%. Bilobed ovary with axial placentation was found in all eight varieties also anomocytic stomata and isopolar pollen was common in the varieties.

Biochemical Variation

Significant variation in capsaicinoids content of the eight *C. annuum* varieties was recorded. The readings were taken in triplicates and results were presented as average value with the standard deviation from the mean. The capsaicinoids content was found in the range of 42.98 ± 0.67 – 126.98 ± 0.23 . The increasing order of capsaicinoids content among the

eight varieties were recorded as *C. annuum* var. Fasciculatum < *C. annuum* var. 'goat horn' < *C. annuum* var. longum < *C. annuum* var. 'Korean long green' < *C. annuum* 'eagle claw' < *C. annuum* var. annuum < *C. annuum* var. conoides < *C. annuum* var. 'cascabel'. Since the capsaicinoids content is regarded as a direct indication of the hotness of the chilly, it was obvious that *C. annuum* var. 'cascabel' is considered as the most pungent variety of *C. annuum* from the north eastern part of India.

Conclusion

This experiment was conducted to point out the distinct morphological and anatomical variations among the eight varieties of *C. annuum* along with its biochemical confirmation

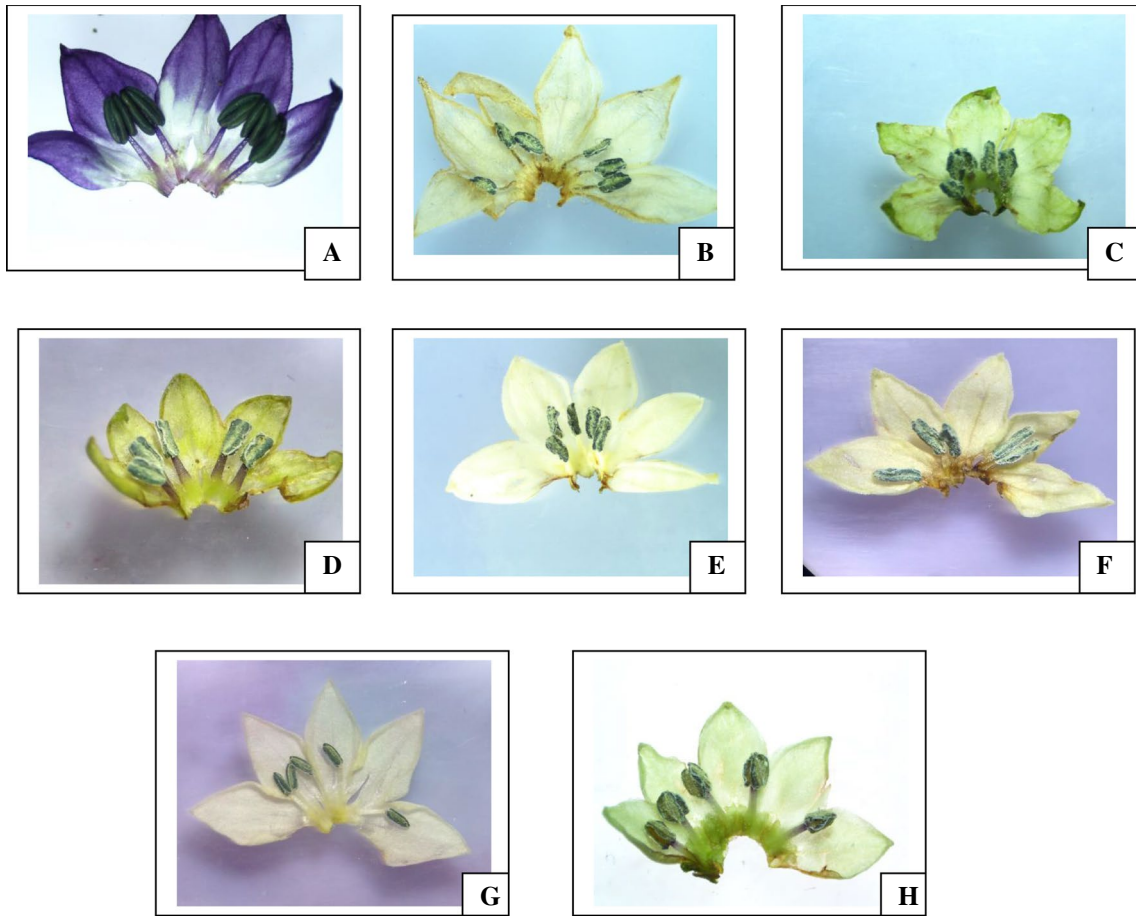


Fig. 3 Flower with Stamen of the eight varieties of *Capsicum annuum* L. **a**—*C. annuum* var. longum; **b**—*C. annuum* var. fasciculatum; **c**—*C. annuum* 'eagle claw'; **d**—*C. annuum* var. annuum; **e**—*C. annuum*

'goat horn'; **f**—*C. annuum* 'Korean long green'; **g**—*C. annuum* var. conoides; **h**—*C. annuum* 'cascabel'

in terms of their capsaicinoids content. Among the morphological specifications, the colour of corolla, size of the flower, colour and shape of the fruit, size of the petioles and leaf provides evidence for variation. The anatomical

variations such as arrangement of vascular bundle of petiole, rings of cortex in stem and stomatal index of both adaxial and abaxial surface of the leaf confirm the existing variation among the varieties.

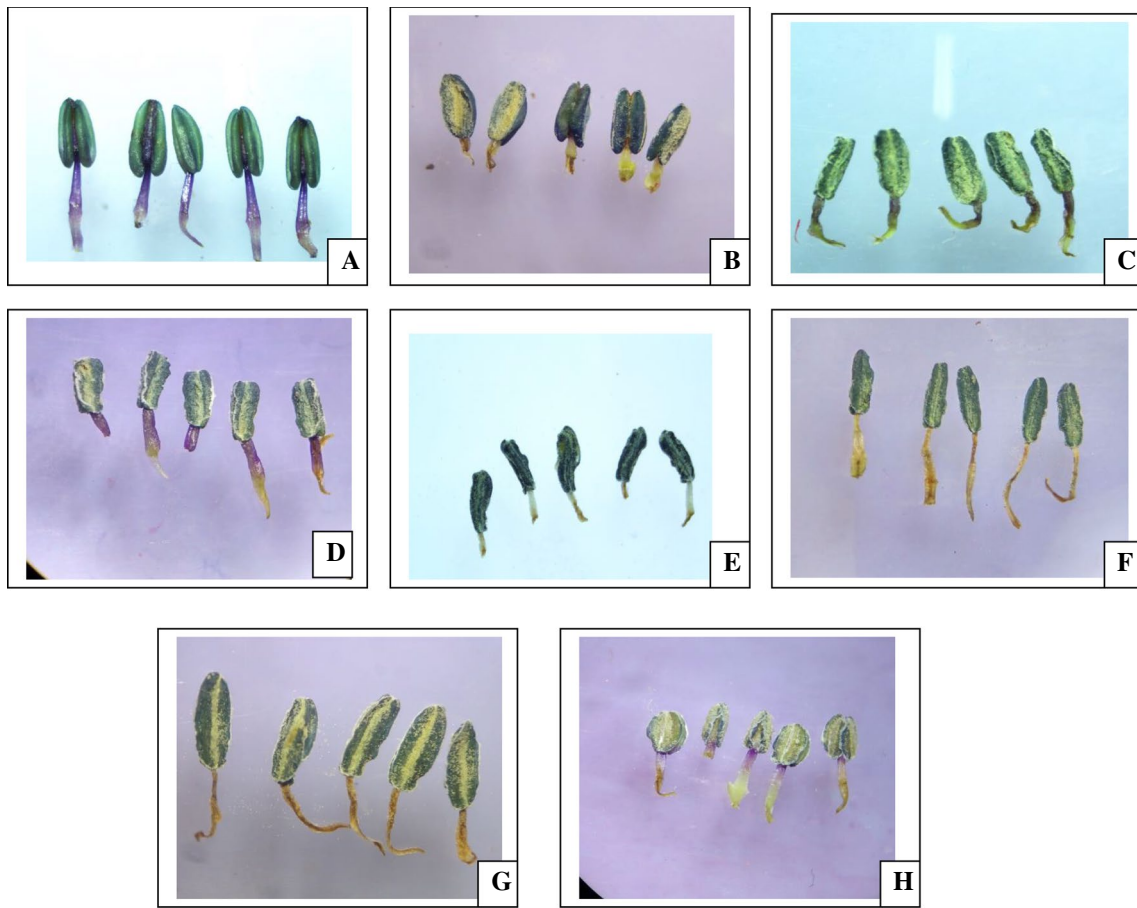


Fig. 4 Stamen of the eight varieties of *Capsicum annuum* L. **a**—*C. annuum* var. *longum*; **b**—*C. annuum* var. *fasciculatum*; **c**—*C. annuum* ‘eagle claw’; **d**—*C. annuum* var. *annuum*; **e**—*C. annuum* ‘goat horn’; **f**—*C. annuum* ‘Korean long green’; **g**—*C. annuum* var. *conoides*; **h**—*C. annuum* ‘cascabel’

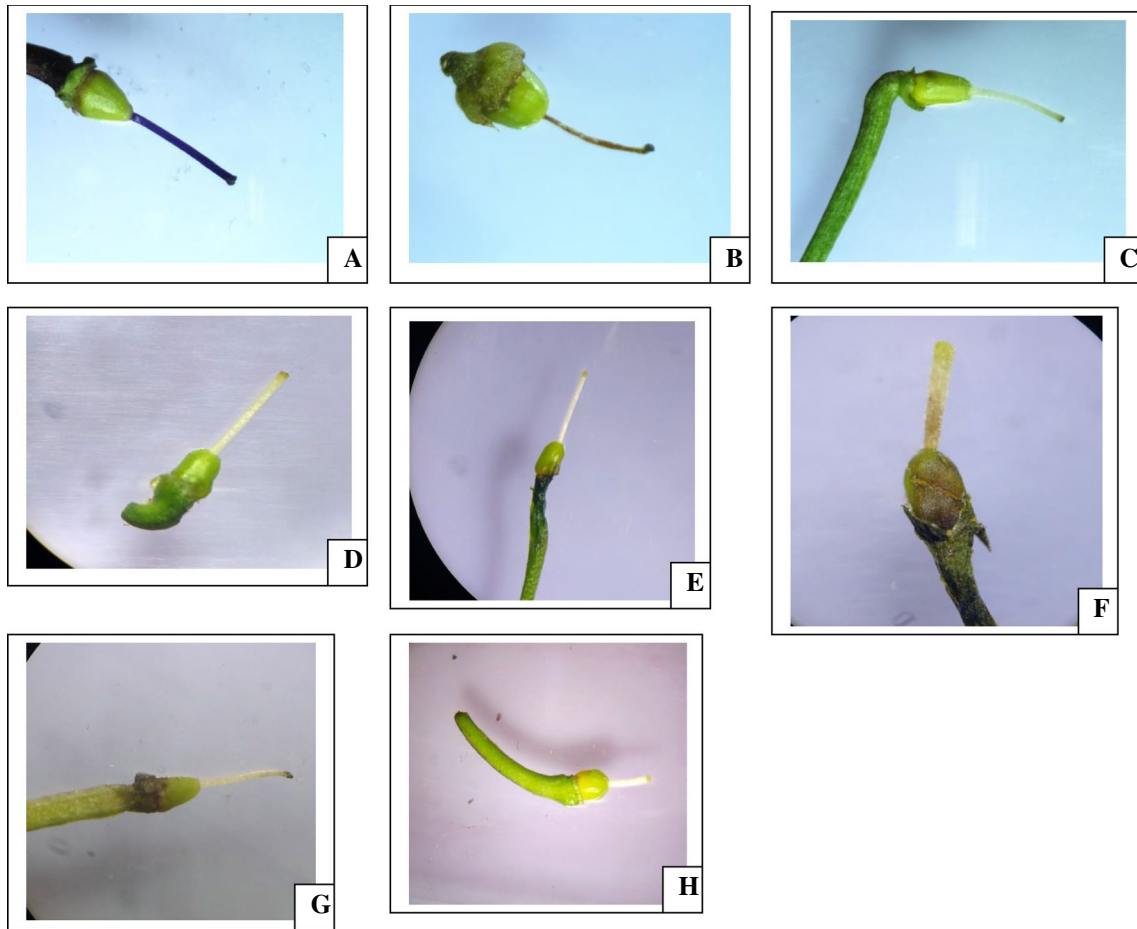


Fig. 5 Gynoecium of the eight varieties of *Capsicum annuum* L. **a**—*C. annuum* var. *longum*; **b**—*C. annuum* var. *fasciculatum*; **c**—*C. annuum* 'eagle claw'; **d**—*C. annuum* var. *annuum*; **e**—*C. annuum* 'goat horn'; **f**—*C. annuum* 'Korean long green'; **g**—*C. annuum* var. *conoides*; **h**—*C. annuum* 'cascabel'

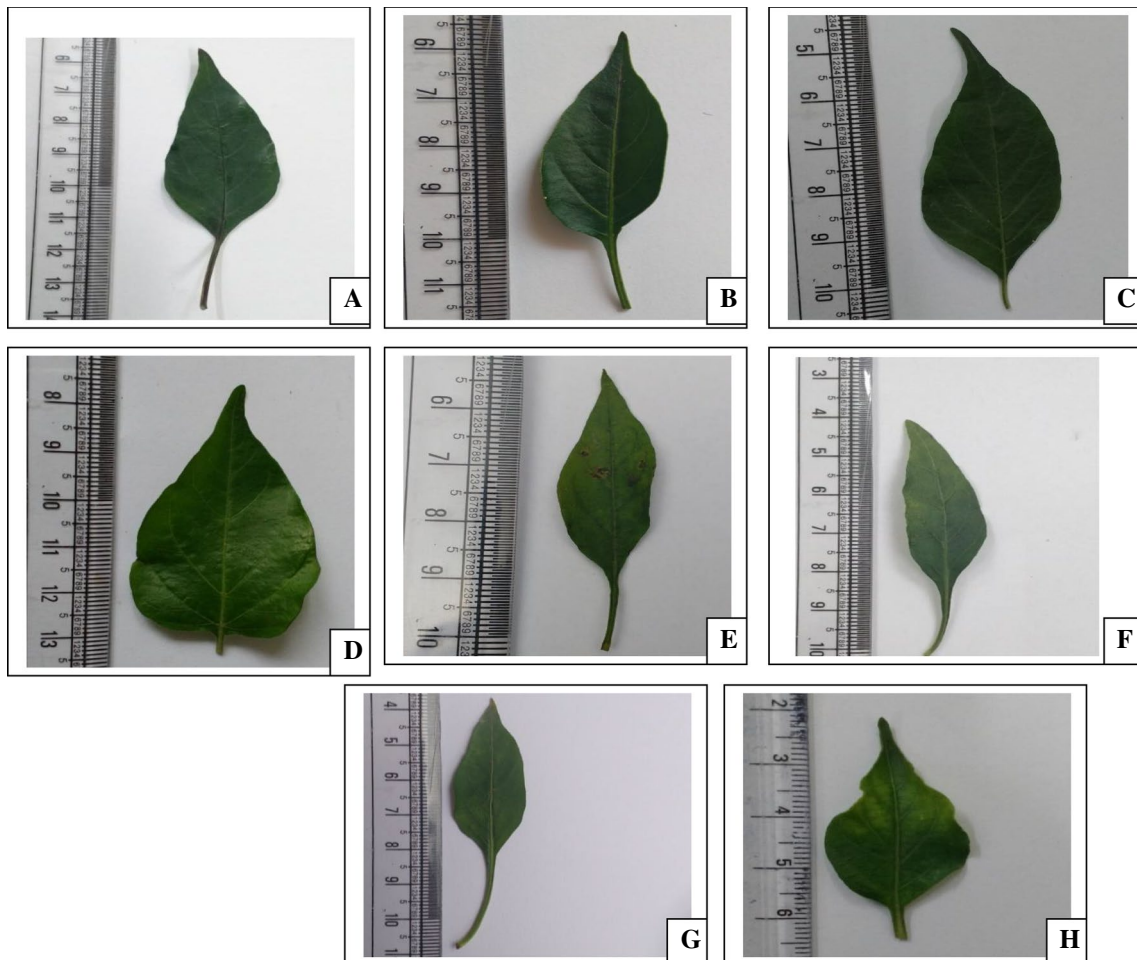


Fig. 6 Leaf adaxial surface of the eight varieties of *Capsicum annuum* L. **a**—*C. annuum* var. longum; **b**—*C. annuum* var. fasciculatum; **c**—*C. annuum* 'eagle claw'; **d**—*C. annuum* var. annuum; **e**—*C. annuum*

'goat horn'; **f**—*C. annuum* 'Korean long green'; **g**—*C. annuum* var. conoides; **h**—*C. annuum* 'cascabel'

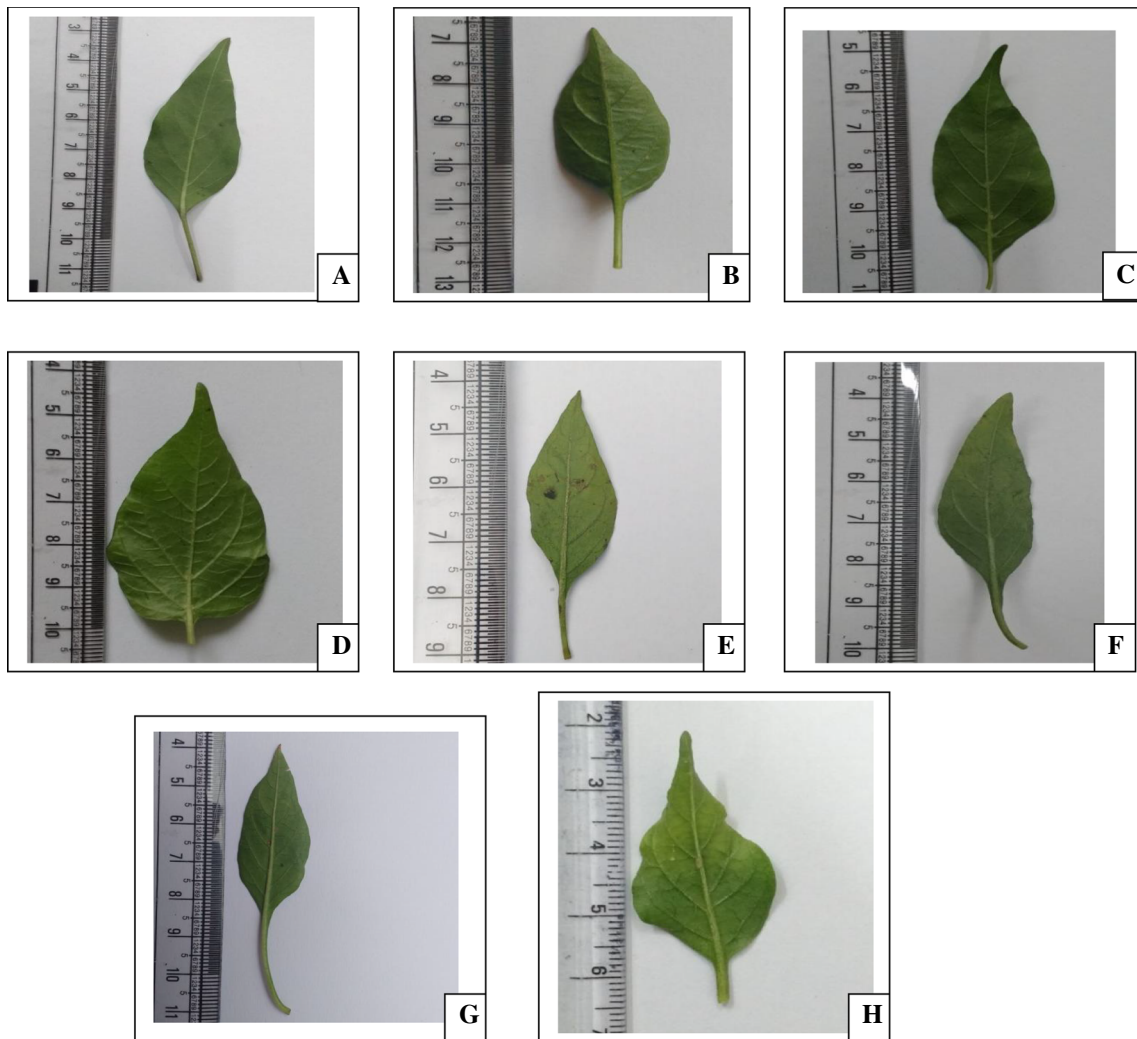


Fig. 7 Leaf abaxial surface of the eight varieties of *Capsicum annum* L. **a**—*C. annum* var. longum; **b**—*C. annum* var. fasciculatum; **c**—*C. annum* ‘eagle claw’; **d**—*C. annum* var. annuum; **e**—*C. annum*

‘goat horn’; **f**—*C. annum* ‘Korean long green’; **g**—*C. annum* var. conoides; **h**—*C. annum* ‘cascabel’



Fig. 8 Fruit of the eight varieties of *Capsicum annum* L. **a**—*C. annum* var. longum; **b**—*C. annum* var. fasciculatum; **c**—*C. annum* 'eagle claw'; **d**—*C. annum* var. annum; **e**—*C. annum* 'goat horn'; **f**—*C. annum* 'Korean long green'; **g**—*C. annum* var. conoides; **h**—*C. annum* 'cascabel'

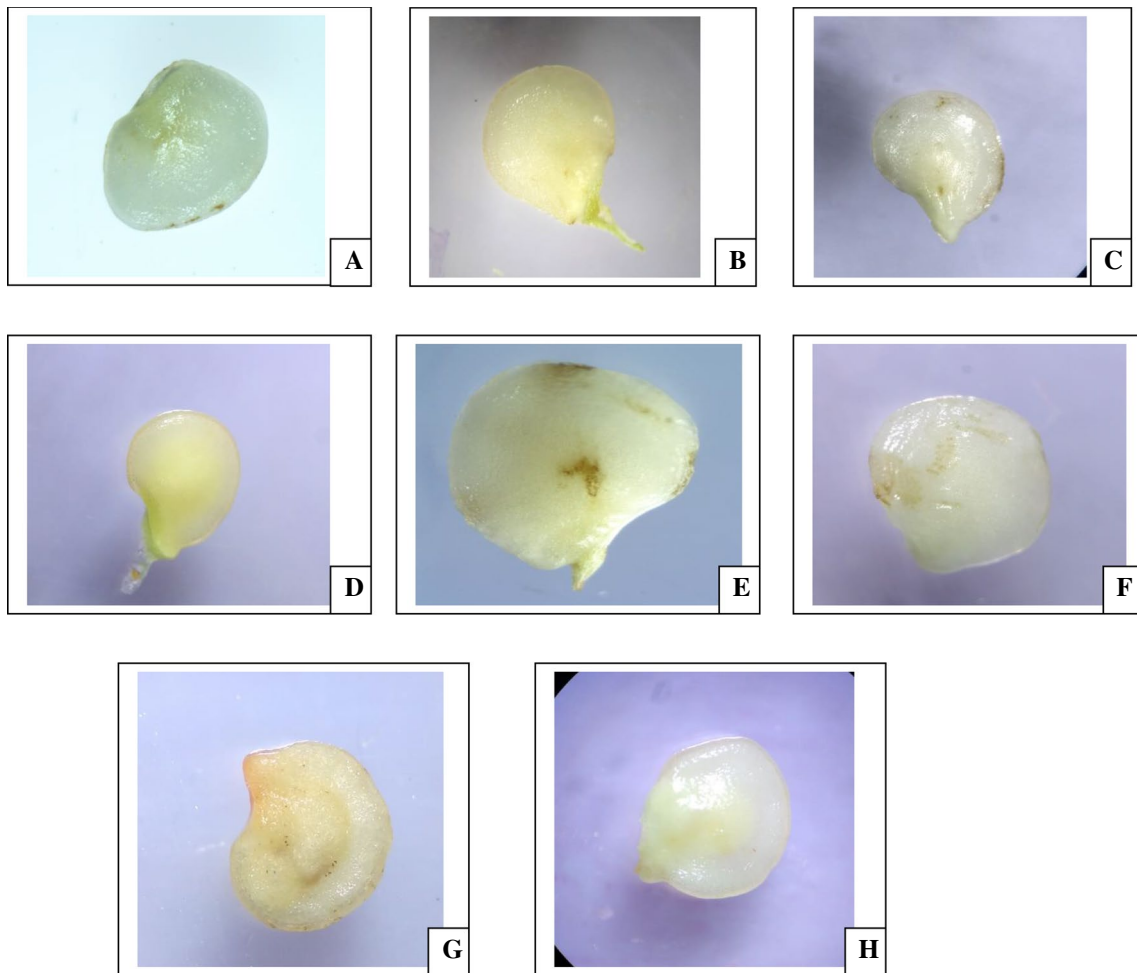


Fig. 9 Seed of the eight varieties of *Capsicum annuum* L. **a**—*C. annuum* var. *longum*; **b**—*C. annuum* var. *fasciculatum*; **c**—*C. annuum* 'eagle claw'; **d**—*C. annuum* var. *annuum*; **e**—*C. annuum* 'goat horn'; **f**—*C. annuum* 'Korean long green'; **g**—*C. annuum* var. *conoides*; **h**—*C. annuum* 'cascabel'

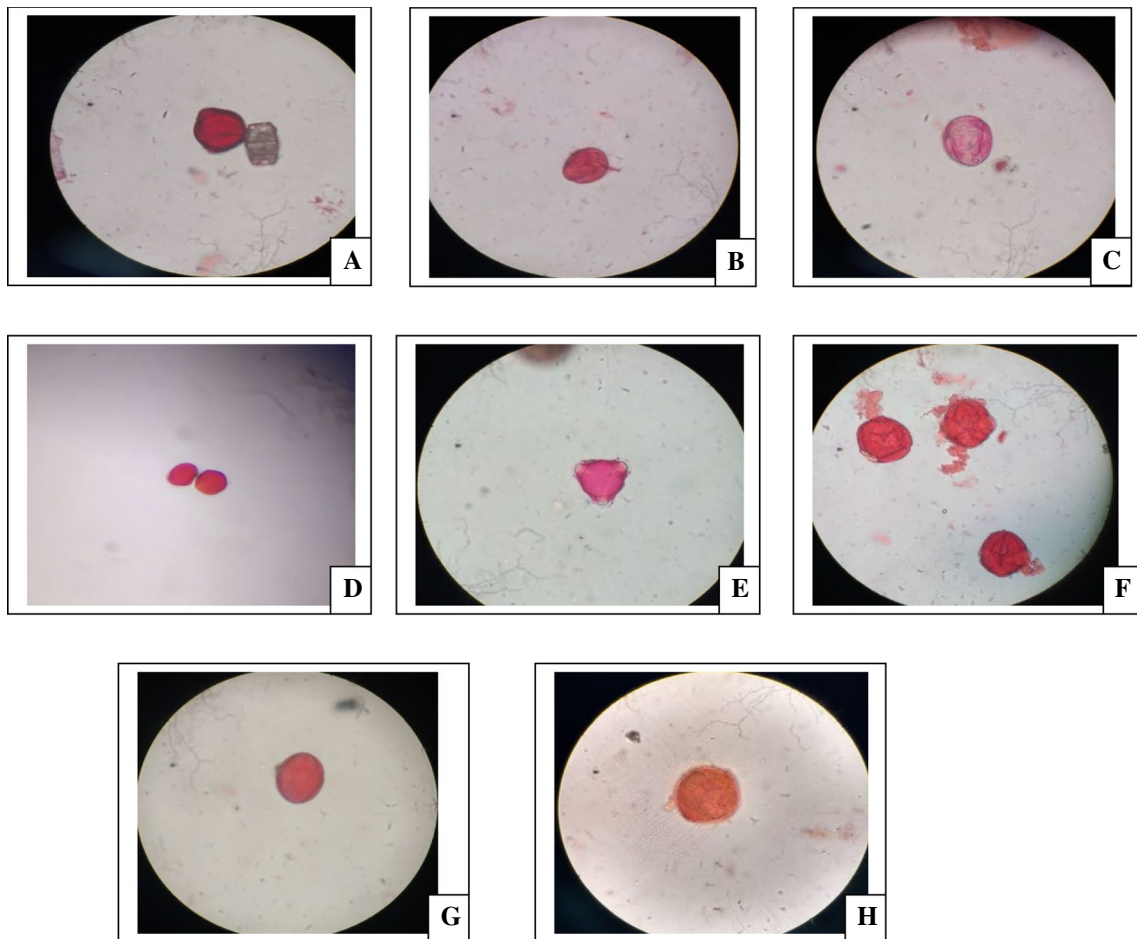


Fig. 10 Pollen of the eight varieties of *Capsicum annuum* L. **a**—*C. annuum* var. *longum*; **b**—*C. annuum* var. *fasciculatum*; **c**—*C. annuum* ‘eagle claw’; **d**—*C. annuum* var. *annuum*; **e**—*C. annuum* ‘goat horn’; **f**—*C. annuum* ‘Korean long green’; **g**—*C. annuum* var. *conoides*; **h**—*C. annuum* ‘cascabel’

Table 2 Anatomical characters among varieties of *Capsicum annuum* L

Varieties	T.s of ovary	T.s of petole	T.s of stem	Pollen	Stomata adaxial with stomatal index	Stomata abaxial with stomatal index
<i>C. annuum</i> var. longum	Axial placentation, bilobed	Crescent, epidermal hair, fragmented vascular bundle	Cortex ring-3, epidermal hair present	Prolate Spheroidal, isopolar	Anomocytic SI= 16%	Anomocytic SI= 18%
<i>C. annuum</i> var. fasciculatum	Axial placentation, bilobed	Crescent, no epidermal hair, non-fragmented vascular bundle	Cortex ring-4, epidermal hair absent	Prolate Spheroidal, isopolar	Anomocytic SI= 3%	Anomocytic SI= 25%
<i>C. annuum</i> 'eagle claw'	Axial placentation, bilobed	Crescent, epidermal hair, non-fragmented vascular bundle	Cortex ring-5, epidermal hair absent	Prolate Spheroidal, isopolar	Anomocytic SI= 12%	Anomocytic SI= 29%
<i>C. annuum</i> var. annuum	Axial placentation, bilobed	Crescent, epidermal hair, c shaped vascular bundle	Cortex ring-3 epidermal hair absent	Prolate Spheroidal, isopolar	Anomocytic SI= 7%	Anomocytic SI= 23%
<i>C. annuum</i> var. 'goat horn'	Axial placentation, bilobed	Crescent, no epidermal hair, non-fragmented vascular bundle	Cortex ring-2, epidermal hair present	Prolate Spheroidal, isopolar	Anomocytic SI= 14%	Anomocytic SI= 23%
<i>C. annuum</i> var. 'Korean long green'	Axial placentation, bilobed	Crescent, epidermal hair, c shaped vascular bundle	Cortex ring-5, epidermal hair present	Prolate Spheroidal, isopolar	Anomocytic SI= 5%	Anomocytic SI= 24%
<i>C. annuum</i> var. conoides	Axial placentation, bilobed	Crescent, epidermal hair, fragmented vascular bundle	Cortex ring-2, epidermal hair present	Prolate Spheroidal, isopolar	Anomocytic SI= 17%	Anomocytic SI= 23%
<i>C. annuum</i> var. 'cascabel'	Axial placentation, bilobed	Crescent, epidermal hair, non-fragmented vascular bundle	Cortex ring-3, epidermal hair present	Prolate Spheroidal, isopolar	Anomocytic NIL	Anomocytic SI= 17%

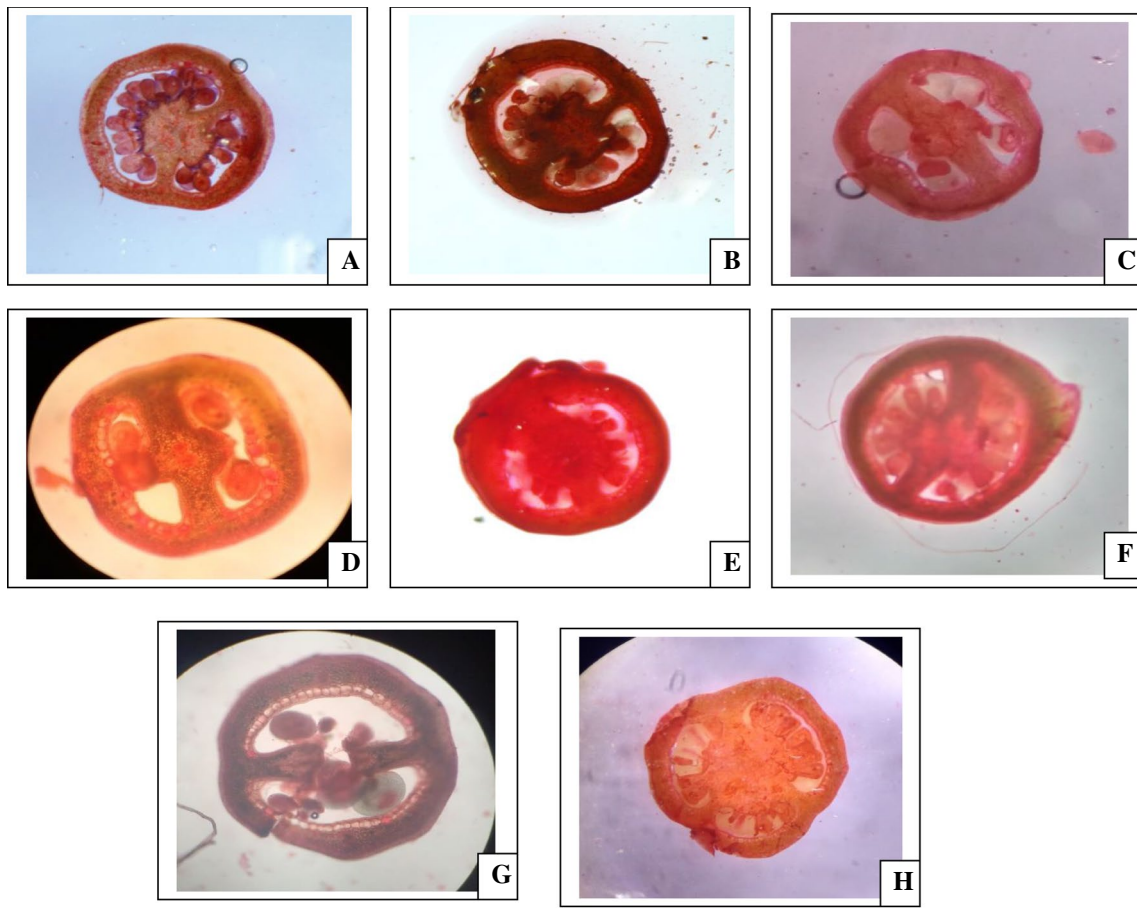


Fig. 11 T.S of ovary of the eight varieties of *Capsicum annuum* L. **a**—*C. annuum* var. longum; **b**—*C. annuum* var. fasciculatum; **c**—*C. annuum* 'eagle claw'; **d**—*C. annuum* var. annuum; **e**—*C. annuum*

'goat horn'; **f**—*C. annuum* 'Korean long green'; **g**—*C. annuum* var. conoides; **h**—*C. annuum* 'cascabel'

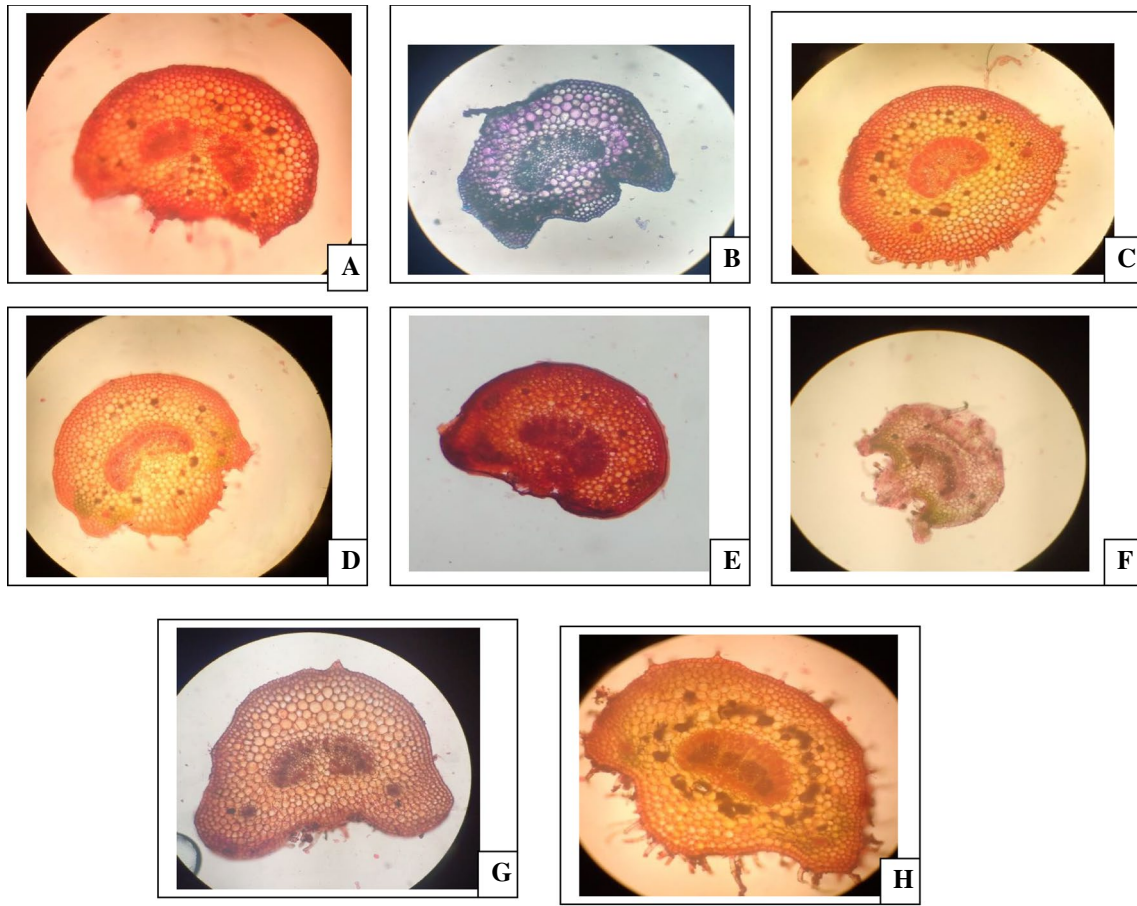


Fig. 12 T.S of petiole of the eight varieties of *Capsicum annuum* L. **a**—*C. annuum* var. longum; **b**—*C. annuum* var. fasciculatum; **c**—*C. annuum* 'eagle claw'; **d**—*C. annuum* var. annuum; **e**—*C. annuum*

'goat horn'; **f**—*C. annuum* 'Korean long green'; **g**—*C. annuum* var. conoides; **h**—*C. annuum* 'cascabel'

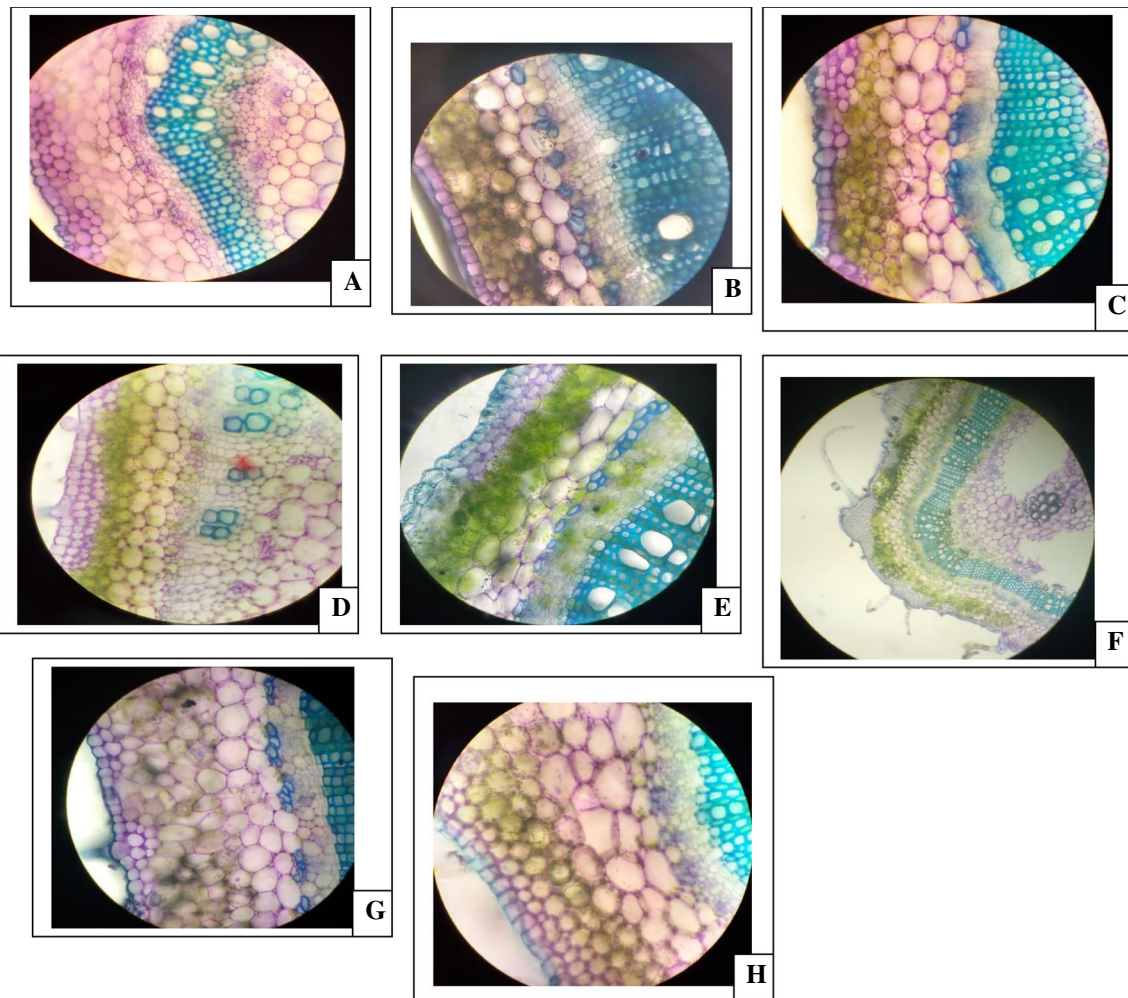


Fig. 13 T.S of stem of the eight varieties of *Capsicum annum* L. **a**—*C. annum* var. longum; **b**—*C. annum* var. fasciculatum; **c**—*C. annum* 'eagle claw'; **d**—*C. annum* var. annuum; **e**—*C. annum* 'goat horn'; **f**—*C. annum* 'Korean long green'; **g**—*C. annum* var. conoides; **h**—*C. annum* 'cascabel'

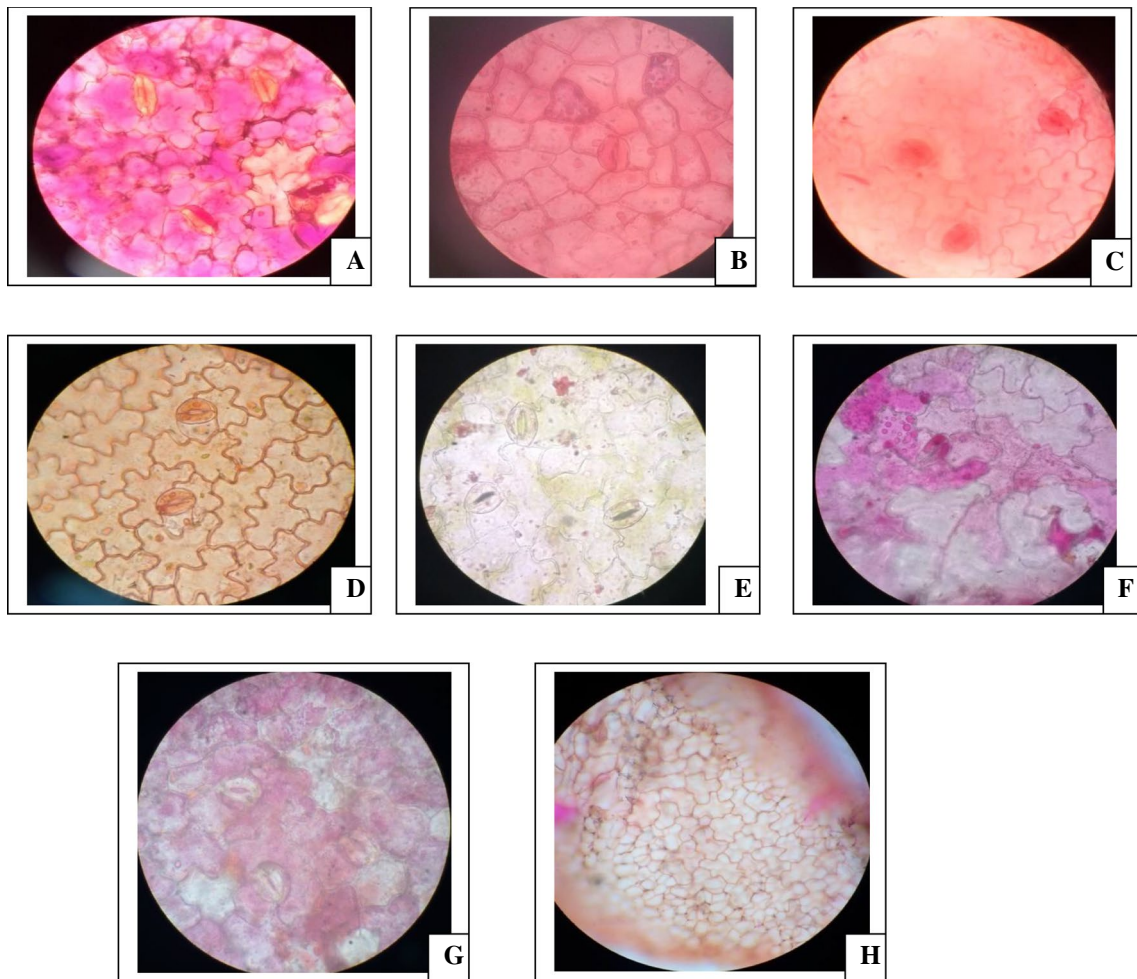


Fig. 14 Adaxial surface of Stomata of the eight varieties of *Capsicum annuum* L. **a**—*C. annuum* var. longum; **b**—*C. annuum* var. fasciculatum; **c**—*C. annuum* 'eagle claw'; **d**—*C. annuum* var. annum; **e**—*C.*

annuum 'goat horn'; **f**—*C. annuum* 'Korean long green'; **g**—*C. annuum* var. conoides; **h**—*C. annuum* 'cascabel'

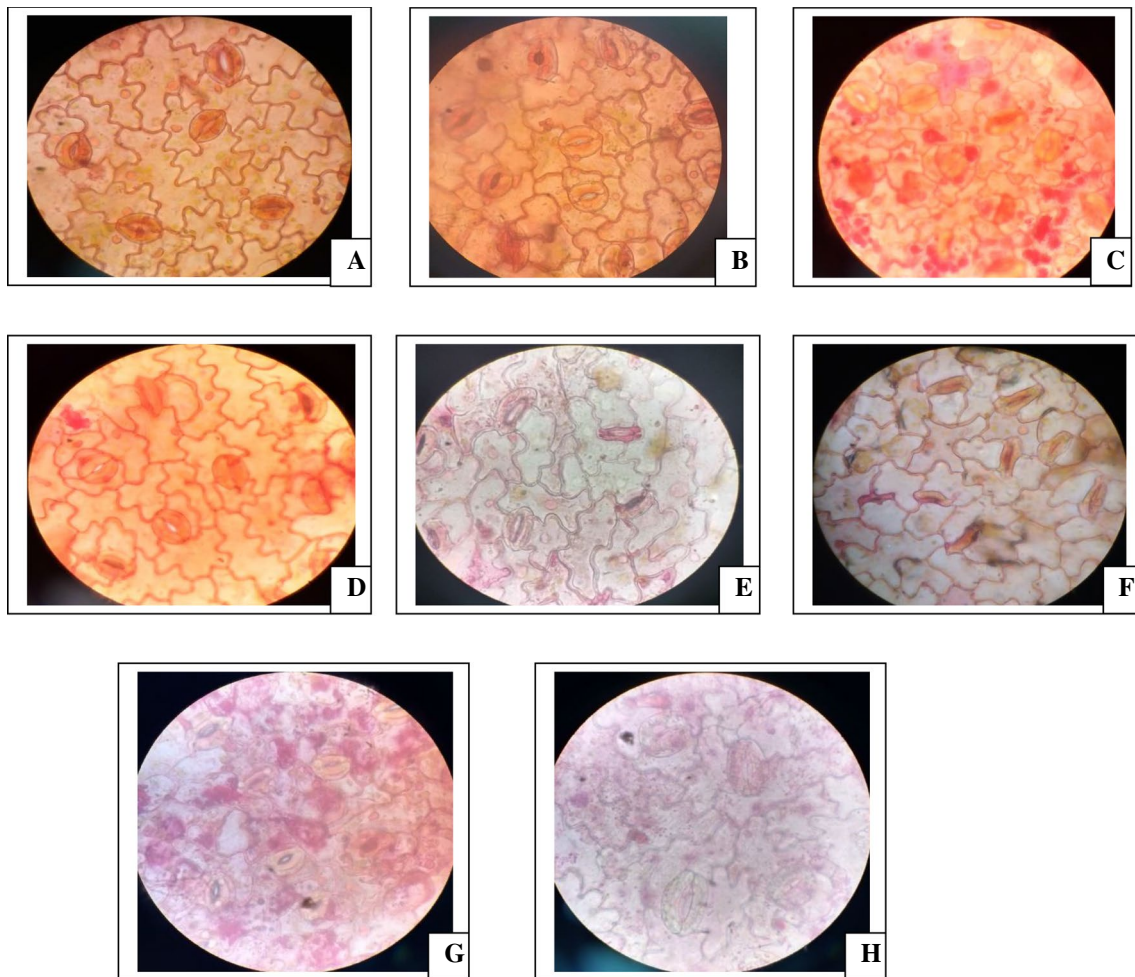


Fig. 15 Stomata of abaxial surface of the eight varieties of *Capsicum annuum* L. **a**—*C. annuum* var. longum; **b**—*C. annuum* var. fasciculatum; **c**—*C. annuum* ‘eagle claw’; **d**—*C. annuum* var. annuum;

e—*C. annuum* ‘goat horn’; **f**—*C. annuum* var.1; **g**—*C. annuum* var. conoides; **h**—*C. annuum* ‘cascabel’

Acknowledgements The corresponding author is thankful to Principal, M.C College, Barpeta for his all the help and guidance in the preparation of this research work and providing the adequate facilities to carry out the research work. The authors have no conflict of interest among each other.

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