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Morphological and anatomical studies in *Nigella sativa* L. (Ranunculaceae)

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Abstract

This research delves into the morphological and anatomical intricacies of *Nigella sativa* L. (Ranunculaceae), commonly known as black cumin, through meticulous examination of various accessions collected from diverse geographical regions in India. Morphological studies revealed distinct characteristics such as the linear to lanceolate alternate leaves, black capsule fruit housing seeds, and growth parameters including germination time, maturation period, and yield per plant. Notably, certain accessions exhibited superior attributes in terms of plant height, root length, and seed yield. Anatomical investigations unveiled detailed structures of stems, roots, seeds, and capsules. Stem anatomy exhibited a multi-cornered structure with specific features like uniseriate epidermis with non-glandular trichomes, parenchymatous cortex with chlorenchymatous and sclerenchymatous cells, and distinct vascular bundle arrangements. Root anatomy depicted a circular cross-section with well-defined layers including epidermis, cortex, and xylem with prominent oil droplets. Seed anatomy displayed layers of epidermis, parenchyma, pigmented layer, endosperm, and a central embryo. Moreover, this study identified novel cells in the stem cortex and observed variability in carpel numbers among different accessions. The findings contribute significantly to the understanding of *N. sativa* morphology and anatomy, shedding light on previously unexplored aspects and emphasizing the need for further investigation into the variability within and among accessions.

Key words: Morphology, anatomy, *Nigella sativa*, Idioblast, Ranunculaceae, capsule, stomata

Introduction

Ranunculaceae is a large angiosperm family of order Ranunculales that is distributed worldwide, especially in the subtropical and temperate regions of the northern hemisphere. This family includes 60 genera and 2346 species, of which 42 genera and about 720 species are distributed throughout China, mostly in the southwest mountainous region (Christenhusz and Byng, 2016). Most of the plants of this family are herbs, some are small shrubs, while others are woody vines. Many Ranunculaceae genera like *Ranunculus, Delphinium, Thalictrum, Clematis* and *Aconitum* are important medicinal plants commonly used in traditional Chinese and Indian medicine systems (Hao, 2019; Ali *et al.*, 2023). Within the eudicots, Ranunculaceae has been regarded as one of the most primitive family of herbaceous angiosperms (Salim *et al.*, 2016).

Nigella is a small genus of family Ranunculaceae and includes around 20 species most of which are annuals with a short life cycle (Salehi *et al.*, 2021). *N. sativa* L. commonly known as black cumin, has played a significant role in traditional medicine, food and culture for centuries in South Asia, the Middle East, Europe, and the Mediterranean. The species is a well- known condiment and spice with significant economic potential, particularly in the food, pharmaceutical and cosmetic industry (Huchchannanavar *et al.*, 2019). Black cumin is used in the preparation of food, pickles, baked goods and several other food items. *N. sativa* is a remedy for fatigue, fever, headache, skin diseases, wounds, fungus, parasites, and poisonous animal bites (Hossain *et al.*, 2021). It grows in Eastern Europe, the Middle East, and Western Asia (Tavakkoli *et al.*, 2017; Hossan *et al.*, 2018). It is an annual flowering plant, characterized by pale blue and white petals and fruit that contain numerous stark black seeds (Hwang *et al.*, 2021). Several phytochemicals like thymoquinone, dithymoquinone, thymohydroquinone, p- cymene, carvacrol, 4-terpineol, α-thujene, t-anethol, longifolene, thymol, and pinene are found in black seed oil (Singh *et al.*, 2015; Rajabian and Hosseinzadeh, 2020). Among its active components, thymoquinone is the most prevalent component of the seed oil and it is the component to which most of the biological activities of this herb are attributed (Almshawit and Macreadie, 2017). Thymoquinone along with thymohydroquinone and carvacrol show activity against a wide range of fungi, bacteria and viruses (Krause *et al.*, 2021; Wang *et al.*, 2021; Pandey *et al.* 2024).

The variation within a species, genera or family is usually reflected in anatomical features due to which the role of anatomy in taxonomy has been widely recognized. Morphological and anatomical traits are considered to be the most important characteristics of Ranunculaceae classification. Apart from this, leaf epidermal traits like stomata, trichomes, along with other anatomical features are also perceived as useful tools in plant systematics (Hao, 2019). Although large number of studies have been conducted on the nutritional and medicinal properties of *N. sativa*, less information is available on the morphology and anatomy in this economically important species. The principal aim of this research is the contribution to the knowledge of *N. sativa* from different points of view *viz.* morphological and anatomical characteristics of stem, flower leaf, seed, fruit and root.

Material and methods

Experimental material: The experimental material included six *N. sativa* accessions from Indian states (Table 1). The Department

of Botany, Mahatma Gandhi Central University, Bihar, sowed the accessions in separate experimental plots and examined their morphological traits at maturity.

Table 1. Details of accessions of black cumin used in the present study

Accession	Source
AN-1	Indian Council of Agricultural Research (ICAR), Rajasthan
AN-20	Indian Council of Agricultural Research (ICAR), Rajasthan
H1	Haryana
O1	Odisha
B1	Bihar
Shyama	RPCAU, Bihar

Micro-morphology: For micromorphological studies, thin sections of the stem, leaves, root, fruit and seed were cut with a microsurgical instrument. The sections were stained in safranin (1% solution in 50% ethanol) and light green (1% solution in 96% ethanol) (Dilcher 1974). The sections were then placed on a drop of 10% glycerol on glass slides, covered with a glass slip, observed under a light microscope and photographed.

Results and discussion

Morphological studies: *N. sativa* is an annual flowering plant bearing linear to lanceolate alternate leaves (Table 2). The fruit was a capsule having black-colored seeds. The accessions took around 10 days to germination (Mean: 10.83+0.96 days) and exhibited synchronous maturity with the maturation period of around 140 Days (Mean: 140+0.97) (Table 3). Among different accession, Shyama exhibited highest values for days to germination (15) and days to flowering (66). The accession AN-1 had highest values for plant height (61.60 cm), root length (14.66 cm), branches/plant (5.3), capsules/plant (6.0) and capsule length (0.9 cm). maximum seed yield/plant was reported for AN-1 (0.621 g/plant), followed by AN-20 (0.474 g/plant) and B1 (0.371 g/plant) (Table 3). The morphological studies revealed the following observations.

Root: Roots of all the investigated plants of *N. sativa* were tap roots. The roots were stout, elongated, cylindrical and branched in all the accessions (Fig. 1 A-F). Similar results were also reported by Hossain *et al.* (2021) who observed that the roots of *N. sativa* were erect, branched and elongated. The roots of AN-1 and AN-20 were stouter than those of other accessions. The root length among the six accessions ranged from 10.00-14.66 cm with a mean of 11.80+0.63 cm (Table 3) with AN-1 exhibiting the highest value for root length (14.66 cm). Thus, the AN-1 accessions had comparatively longer root length in comparison to the other accessions.

Stem: The stem was aerial, long, shiny green and hollow in all the examined accessions. The stem length ranged from 43.90-61.60 cm (Table 3), and had highest values for local accessions as was the case with root length. Intense branching was observed at the base as well as on the upper portion in the local accession B1. However, the rest of the accessions showed branching only in the upper portions. The stem was herbaceous, cylindrical and pubescent with glandular hairs. The stem of accessions originated from Rajasthan *viz.*, AN-1 and AN-20 were erect and quite strong as compared to those from other states. The texture of stem was

Table 2. Agro-morphological traits of *Nigella sativa*.

Traits	Characteristics
Stem	
Diameter (cm)	0.65-0.98
Shape	Angular
Texture	Smooth and hairy
Leaves	
Arrangement	Alternate
Length (cm)	2.5-5.0
Width (mm)	2.0-2.5
Shape	Linear to lanceolate
Phyllotaxis	1-2
Pinna/rachis	5-6
Flower	
Colour	White or pale blue
Size (cm)	2.74 × 2.78
Number of petals	5-8
Number of sepals	5
Seed	
Capsule size (cm)	0.6-1.1
Colour	Black
Seed length (mm)	2-3
Shape	Acute, angles sharp, ovate, tetra-angular, more tapering at the end.
Flowering and fruiting time	January to April

Fig. 1. Variation in root morphology in different accessions of *N. sativa*. A- Odisha; B-AN-20; C- Shyama; D-; Haryana; E- AN-1; F- Bihar

Table 3. Morphological traits in six accessions of *Nigella sativa*

Variety	Days to germination	Days to flowering	Days to 50% maturity	Stem length (cm)	Root length (cm)	Branches/ plant	Capsules/ plant	Seeds/ capsule	Capsule length (cm)	Seed yield (g/plant)	HI
AN-1	10	58	138	61.60	14.66	5.3	6.0	52	0.9	0.621	0.511
AN-20	13	61	143	57.40	12.40	5.0	5.0	40	0.9	0.474	0.630
Haryana	9	54	142	43.90	12.10	3.66	3.33	40	0.8	0.334	0.794
Odisha	9	55	141	46.86	11.21	3.0	2.6	30	0.7	0.236	0.196
Bihar	9	55	136	47.96	10.00	4.6	3.66	57	0.8	0.371	0.351
Shyama	15	66	140	44.63	10.43	2.3	2.66	32	0.6	0.158	0.162
Range	9-15	54-66	136-143	43.90-61.60	10.00-14.66	3.0-5.3	2.6-6.0	30-57	0.6-0.9	0.158-0.621	0.162-0.794
Mean+SE	10.83±0.96	58.17±1.72	140±0.97	50.39±2.73	11.80±0.63	3.98±0.44	3.88±0.51	41.83±4.00	0.78±0.04	0.37±0.06	0.441±0.093

smooth and hairy in all the accessions. All the plants had sympodial branching which was bilateral. Similar results were reported by Rashid *et al.* (2018) and Thilakarathna *et al.* (2018) who found that the stem of *N. sativa* was erect, branched, long and striate.

Leaves: The leaves were petiolate, exstipulate, tripinnate compound with unicostate reticulate venation in all the accessions under study. Leaves were ramal and cauline with alternate arrangement of leaves. Leaves were filiform that is with long and slender petiole in all the plants. The shape of leaves was linear, acute, entire, dark green, and finely divided by thread like and wispy lobes of 0.8-2 mm width in all the examined plants. The surface of leaf was glabrous and hairy in all the examined plants. The colour of leaf midrib was green and the lateral venation of leaf was not distinct in any of the plants under study. These results were in consonance with those reported by Sultana *et al.* (2015), Rashid *et al.* (2018) and Hossain *et al.* (2021) who mentioned that the leaves of *N. sativa* were exstipulate, branched, finely divided leaves, acute and linear.

Flower: The flowers were delicate and pale green when young and light blue when mature, and later became pale blue (Fig. 2 A) and white in color (Fig. 2 B) (Table 2). White flowers were usually more in numbers then pale blue flowers. Flowers were cyclic, hypogynous, solitary, hermaphrodite or bisexual, pedicellate, ebracteate, actinomorphic and complete in all the accessions (Fig. 2 A). Sepals were five in number, petaloid, polysepalous and with valvate aestivation. The shape of sepals was lanceolate, entire and acute. Corolla was pale blue at base, green at centre and white at the top with pointed end. Petals were smaller than sepals, stalked, bilabial, with outer labium modified into bifid nectaries. The margin of petals was surrounded by white colour appendages. The flowers had five or more petals (upto 8) (Table 2), short, thick, gamopetalous, and with valvate aestivation. Similar results were reported by Sultana *et al.* (2015) and Diwakar *et al.* (2018) who observed that the flowers were white and pale purple with 5 petals. Stamens were numerous in number, polyandrous, monothecous, adnate and with long filament. The position of stamens was exserted. The dehiscence of anther takes place via latrorse. Stamens were arranged in a single whole in all the examined plants. Gynoecium, the female reproductive part, was elongated, partially united at the base and free above, multicarpellary, apocarpous, multilocular, with axile placentation and superior ovary. Style was long and terminal, while the stigma was bifid and persistent. Maximum variation in carpel number was observed in plants from Bihar (2-17 carpels) (Fig. 4), while plants from other locations usually had 3-6 carpels.

Fig. 2. Different flower color types in *N. sativa*: A- Pale blue; B- White

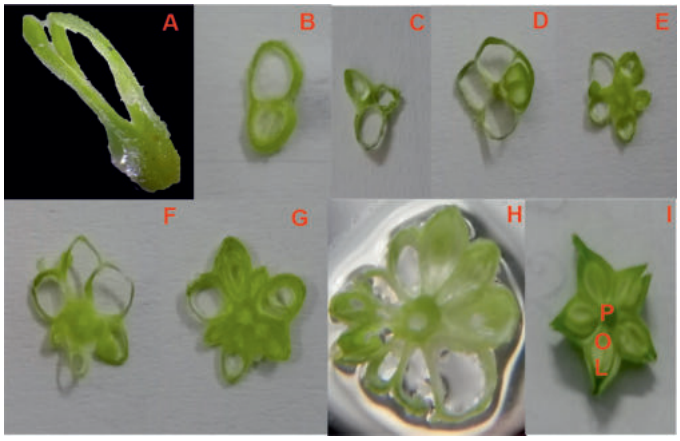

Fig. 3. Female reproductive organs in *N. sativa*. A: Ovary; B-I: T.S. of ovary. OW-Ovary wall; P- Placenta; O- ovule; L- Locule.

Fruit: The fruit was a large and inflated capsule composed of usually three to six united follicles. Plants from Bihar had 2-17 united follicles (Fig. 4). Capsules were green when young and turned pale yellow on maturity. Each capsule contained approximately 20-60 seeds. Maximum seeds/ capsules were observed in AN-1 (60-65). Similar findings were reported by Sultana *et al.* (2015) and Ali *et al.* (2020) who found that fruits of *N. sativa* were large, inflated, and contained several seeds. The average length and width of capsules obtained from plants of Bihar, Haryana, Odisha, AN-1, AN-20 and Shyama ranged from 0.8-0.7 cm, 0.9-0.8 cm, 0.8-0.8 cm, 1.0-0.9 cm, 0.9-0.8 cm, and 0.7-0.6 cm, respectively.

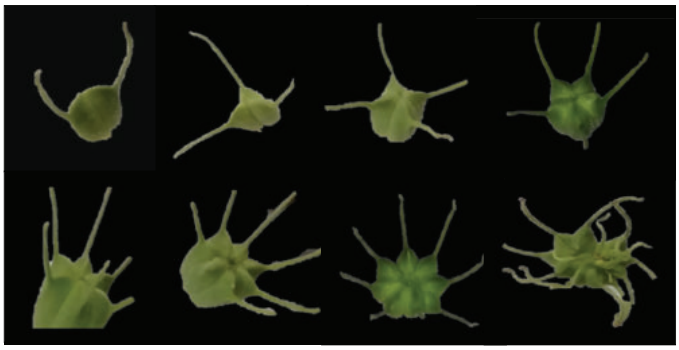

Fig. 4. Variation in carpel number in *N. sativa*.

Seed: Seeds were small dicotyledonous, trigonous, rugulose-tubercular with rough surface. Seeds were black, roughly triangular or pyramidal in shape, 1.5-3 mm long, borne in a capsule usually with 3-6 segments, each of which terminated in an elongated projection. The seeds were black externally but oily white inside, with slightly curved and tapering ends. The seeds were bitter in taste, aromatic and peppery with a crunchy texture. Similar seed properties were reported by Sultana *et al.* (2015), Thilakarathna *et al.* (2018), Eltahir *et al.* (2020) and Hossain *et al.* (2021) who mentioned that seeds were trigonous, rough surface with black exterior and white inner interior.

Anatomical observations

Stem anatomy: Stem had a multi-cornered structure as had also been observed in several other species of the genus like *N. latisecta, N. arvensis, N. lancifolia, N. nigellastrum, N. unguicularis, N. elata* and *N. damascena* (Kökdil *et al.*, 2006). The radial longitudinal section of the region showed the presence of simple pits. Stem was further divided into the following parts:

Epidermis: Outline of section was circular with wavy margin in all the examined accessions (Fig. 5 A). Epidermis was uniseriate, single layered, radially tangential consisting of compact thin-walled cells and covered with thick cuticle (Fig. 5 B-C). A large

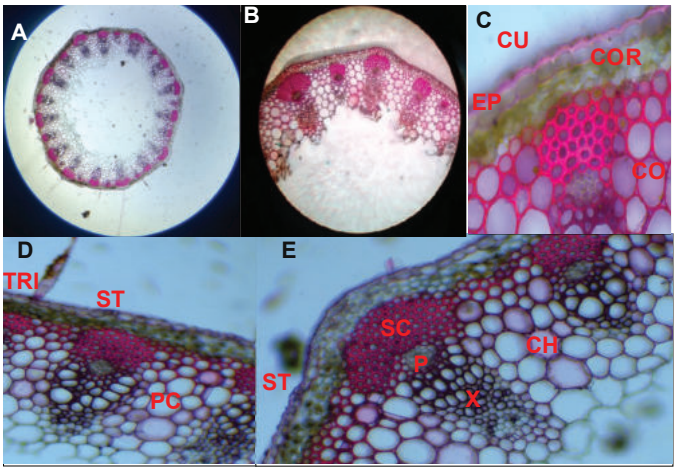

Fig. 5. A: Outline of stem; B: Epidermis; C. Cuticle; D. Trichome; E. Chlorenchyma. CU-Cuticle; EP- Epidermis; Cor- Cortex; CH-Chlorenchyma; CO- Collenchyma; Tri- Trichome; ST- Stomata; PC-Parenchyma cell; SC- Sclerenchyma cell; P- Phloem; X- Xylem

number of unicellular, unbranched hairs known as trichomes arose from the epidermis. Trichomes were non- glandular, simple, long with broad base tapering at the apex (Fig. 5 D). A few anomocytic stomata were also seen in the transverse section (Fig. 5 D-E). Salim *et al.* (2016) and Rashid *et al.* (2018) have also reported angular outline of the stem, with thick cuticle and non-glandular trichomes having broad base and tapering ends.

Cortex: Beneath the epidermis lay the cortex which consisted of 1-3 layers of thin zone of parenchymatous cells (Fig. 5 C). Few chlorenchymatous cells (Fig. 5 E) were present along with collenchymatous cells (Fig. 5 C) in the interfascicular area of vascular bundles in all the examined accessions. Vascular bundles present at the corners were larger than those located in other regions (Fig. 5 B, E) There was a large and thick bordered sclerenchymatous layer (Fig. 5 C-E) on the phloem in all the examined plants. Presence of collenchymatous and sclerenchymatous cells in the cortex has earlier been reported in *N. sativa* by Salim *et al.* (2016). On the underside of vascular bundles, pith was present during the initial period which disappeared at the end of the season. Pith was large, filled with parenchymatous cells having intercellular spaces and hollow in all the accessions (Fig. 5 A, B). The presence of parenchymatic cells was confirmed between the vascular bundles (Fig. 5 D). Three to five novel cells (NCs) were observed in the parenchymatous area, especially in the interfascicular region (Fig. 6 A-D). These may be secretory cells that are associated with the accumulation of metabolic byproducts which are not used as reserve substances (Buvat, 1989). Presence of secretory cells has also been earlier reported in other species of *Nigella* like *N. orientalis*, *N. oxypetala*, *N. arvensis* and *N. elata* (Kökdil *et al.*, 2006). However, the exact nature of these cells needs further detailed studies and validation.

Vascular bundles: Cross section of stem showed the presence of 18-26 vascular bundles situated towards the periphery of the stem in all the examined accessions (Fig. 5 A). The vascular bundles were collateral, closed, endarch and arranged in a single layer. Phloem cells were compactly arranged and were polygonal in shape (Fig. 7). Cambium ring was absent between phloem and xylem. A patch of hard sclerenchymatous tissue known as bundle cap was present outside the phloem of the vascular bundle (Fig. 7). The vascular supply showed continuous siphonostele in all the examined plants similar to that reported earlier by Salim *et al.* (2016).

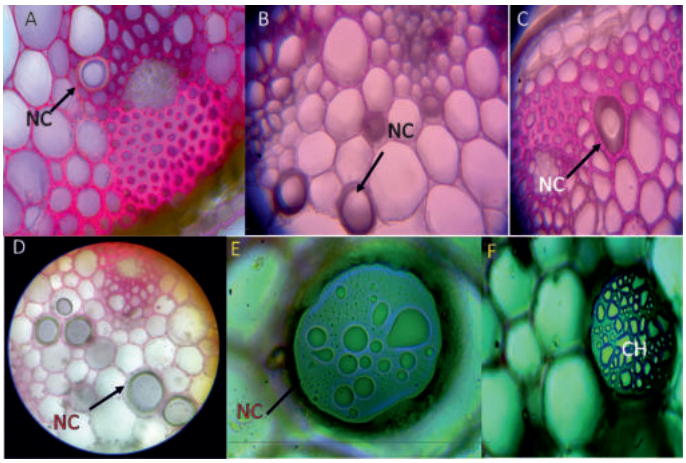

Fig. 6. Specialized cells found in black cumin stem. A-E: Novel cells (NC); F: Chlorenchyma

Fig. 7. Transverse section of *N. sativa* stem showing vascular bundles. P- Phloem; BC- Bundle Cap

Root: Outline of cross section of *N. sativa* root was circular in all the examined accessions (Fig. 8 A). The layer from outer to inner included the epidermis that consisted of 1-3 layers of rectangular cells arranged adjacently. Cortex consisted of few layers of parenchymatous polygonal, small sized, thin-walled cells having intercellular spaces. There were loosely arranged rectangular idioblasts inside the cells. Oil droplets were present throughout the xylem in large amount (Fig. 8 B-C). Xylem occupied comparatively larger area than phloem. Phloem and pith had small parenchymatous cells with intercellular spaces (Fig. 8 B-C).

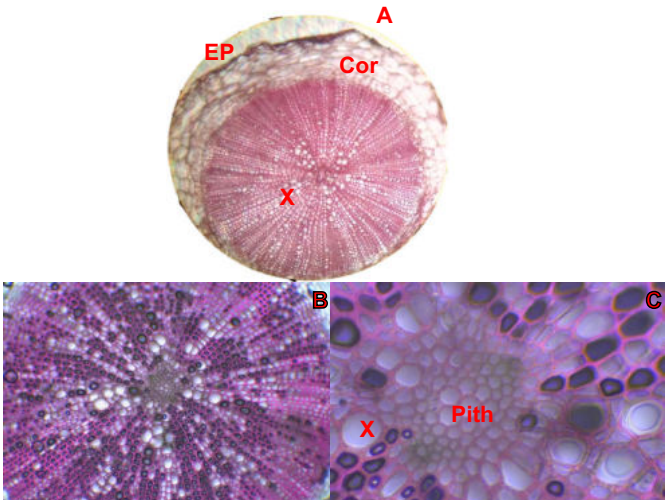

Fig. 8. Transverse section of *N. sativa* root. A- Outline section of root; EP-Epidermis; Cor- Cortex; X- Xylem.

Seed: The cross section of the seed showed a single layered epidermis consisting of elliptical, thick-walled cells, covered externally by a papillose cuticle and filled with dark brown contents (Fig. 9). Epidermis was followed by 2-3 layers of thick walled tangentially elongated parenchymatous cells, followed by a reddish-brown pigmented layer composed of thick walled, rectangular elongated cells in all the accessions. Inner to the pigmented layer was present a layer composed of thick walled rectangular elongated or nearly columnar, elongated cells. Endosperm consisted of thin walled, rectangular or polygonal cells with interspersed oil globules (Fig. 9). A tiny embryo was located in the central region of the section. Eltahir *et al.* (2020) and Sharma *et al.* (2011) also reported that epidermis was covered with thick wall of papillae.

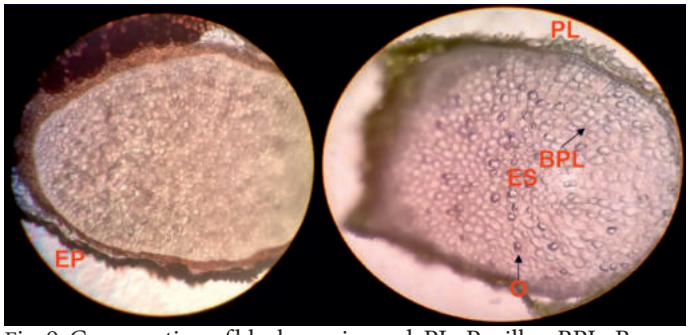

Fig. 9. Cross section of black cumin seed. PL- Papillae; BPL- Brown pigment layer; EP- Epidermis; ES- Endosperm; O- Oil droplets.

Stomata: Leaves of *N. sativa* were amphistomatic *i.e.*, having stomata on both the surfaces. The stomata were anomocytic and surrounded by a limited number of epidermal cells in all the examined accessions (Fig. 10 A-B). Epidermal cells were irregular in shape with undulating margins. Stomata were large, oval in shape, present in large numbers and unequally distributed throughout the leaf in all the accessions. Stomata consisted of two guard cells with a thin outer and thick inner wall (Fig. 10 A-B). Similar results were obtained by Salim *et al.* (2016) and Rashid *et al.* (2018) who found anomocytic stomata with irregular epidermal cells in *N. sativa*.

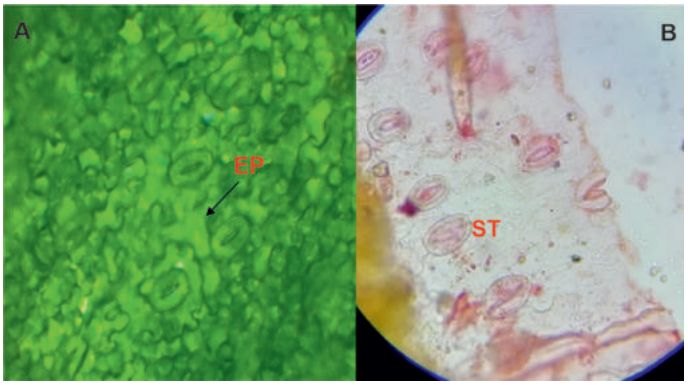

Fig. 10. A. Basic cells of leaf epidermis; B. Stomata; EP- Epidermis, ST- Stomata

Capsule: We report for the first time the transverse section of capsule from basal region in black cumin. The epidermis was wavy and composed of a single layer of rectangular compressed cells. The vascular bundles were arranged in a definite ring.

This study provides the first detailed description of the morphological and anatomical characteristics of *N. sativa* germplasm collected from different geographical locations and agroclimatic regions of India. The current investigation throws light on several untouched aspects of *N. sativa* anatomy and reports for the first-time certain novel cells in the cortex region and variability in the carpels both within and different accessions that need to be further investigated.

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