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# Morpho-Physiological Characterization of Seeds and Seedlings of *Nigella sativa* Linn.: Study on Indian Germplasm

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## Abstract

*Nigella sativa germplasm, collected from 10 different states of India was characterized for morpho-physiological traits of seeds and seedlings. Seeds exhibited variation with respect to shape, color, size and weight. Seeds from Delhi, Kerala, Uttar Pradesh and Sikkim exhibited variation in shape varying from bulging to pointed (proximal and distal) ends. Color of seed also exhibited variation ranging from davy's grey to jet black. Largest seed size was exhibited in seeds of Punjab (3.01±0.0216 mm in length and 2.0±0.081 mm in width), while minimum size was exhibited by seeds of Uttarakhand (2.83±0.021 mm in length and 1.6± 0.081mm in width). Maximum average seed weight was observed with the seeds from Kerala (2.40±0.016 g), while minimum was observed with seeds of Uttarakhand (2.16±0.024g). The respective seedlings also exhibited variation. In the seedlings, maximum root and shoot length was observed with sample from Karnataka (3.3±0.16 cm and 6.1±0.85 cm, respectively), while minimum was observed with sample from Rajasthan (1.6±0.29 cm and 3.5±0.54 cm, respectively). Maximum leaf area (12.06±0.17 mm$^2$) was observed in seeds from Delhi while a minimum was observed with seeds of Punjab (7.83±0.139 mm$^2$). Among physiological parameters, maximum fresh weight and dry weight ratio was found in seedlings from Uttarakhand (24.10±2.029) and minimum was observed in that of Kerala (12.47±2.380). Chlorophyll a and b were found maximum in seedlings from Sikkim (8.21±0.495µg /gram fresh weight), and Jammu and Kashmir (4.61±1.04µg / gram fresh weight), respectively, while minimum was observed with seedlings from Delhi (6.58±0.665µg / gram fresh weight) and Sikkim (3.28±0.20µg / gram fresh weight), respectively. When analyzed for carotene content, highest amount of total carotene was found in seedlings from Uttar Pradesh (1045.19±134.88µg / gram fresh weight) and minimum in seedlings from Rajasthan (799.80±56.52µg / gram fresh weight). Two way ANOVA also supported significant variation among the germplasm (P- value < 0.05). The parameters studied here, indicated variation in the chemical constituents of seeds and their respective seedlings. It would be interesting to see whether this study may be extended to look into further biochemical and genetic variability.*

**Keywords:** Carotene, chlorophyll, morphological characterization, *Nigella sativa*, physiological characterization.

## Introduction

The genus *Nigella* (family Ranunculaceae) comprises of 14 species of annual herbaceous plant of Mediterranean region and Western Asia. It includes species of commercial significance as spices, fragrant, therapeutic and ornamental plants[1]. The plant is cultivated in India, Bangladesh, Turkey, Middle-east and the Mediterranean basin chiefly for its seed or "black cumin" which is almost completely used for edible and therapeutic purposes, such as spices and for cure of a variety of ailments. The components of the *Nigella sativa* seeds have been shown to have immense therapeutic value as revealed to possess diuretic[2], cholagogic, antispasmodic[3], carminative[4], galactogogic[5], antifungal[6], anthelmintic[7] and antidiabetic[8] properties. In Indian system of remedy, seeds are also used as astringent, emmenagogue, hepatoprotective, antiparalytic, antipyretic and to cure skin ailments[9]. It also possesses antitumor, CNS depressant, anticonvulsant, antiurolithiatic and immuno-modulatory activities[10].

Many of the phytochemicals found in *Nigella sativa* have been reported to possess significant antioxidant capacities that have been linked with lesser mortality rates of cancer patients[11]. Nigellone, a chemical component of *Nigella sativa* has exhibited potential consequence on enhancing ciliary clearance, thereby easing asthma patients[12]. Thymoquinone, an important antioxidant, has been reported to inhibit tumor angiogenesis and tumor enlargement through suppressing activation of AKT (protein kinase B) and extracellular signal-regulated kinase signaling pathways[13].

Nigella species have been investigated phytochemically and pharmacologically, but morphology and physiology of this genus and variability there in, still remains to be explored. The present study thus focuses on morphological and physiological aspects of *Nigella sativa* cultivar growing in India. The study is based on the findings that survival and subsequent development (after germination) of the next generation depends on seed parameters such as size[14]. Similarly, the shape, color and average weight are also important as these are manifestations of stored food material and secondary metabolites available in seeds.

## Material and Methods

**Plant materials:** Considering India's vast geographical and climatic conditions, seeds of *Nigella sativa* were collected from different locations in India, representing different climatic and edaphic zones viz. Delhi, Jammu and Kashmir, Karnataka, Kerala, Maharashtra, Punjab, Rajasthan, Sikkim, Uttarakhand, Uttar Pradesh. Ajmer Nigella (AN1) was obtained from National Research Centre on Seed Spice, Tabiji, Rajasthan, India, as reference material. The seeds were thoroughly washed and incubated on sterile wet cotton pads for germination under identical conditions for humidity, temperature and photoperiod. The dry seeds as such or seedlings obtained after 10 days of germination were used in this study.

**Morphological characterization:** The collected seeds were used to study variation in morphological features specially color, shape, size and weight of seeds. For color and shape the seeds were examined under compound binocular microscope (Magnus MLX) and photographed using digital camera.

**Physiological characterization:** The seedlings obtained after 10 days of germination were then studies for traits like average root/ shoot length and average leaf area. Fresh weight and dry weight ratio of the seedlings was calculated by taking the average weight of 10 randomly taken seedlings. Dry weight was taken after incubating the seedlings at $72^0$C for 18 hrs. Chlorophyll a, chlorophyll b and total carotene content was determined spectrophotometrically, with some modifications in in protocol of Dere et al.[15]. The weighed samples were homogenized in 100% acetone. The homogenate was filtered through Whatman no.1 filter paper, and was centrifuged using the REMI cooling centrifuge (Model C-24BL) at 2500 rpm for ten minutes. The supernatant was collected and the absorbance was taken at 662 nm (for chlorophyll a) 645 nm (for chlorophyll b) and 470 nm (for total carotene) using Schimadzu UV- 1800 spectrophotometer and calculated according to the formulae mentioned below[16].

Chlorophyll a = 11.75 $A_{662}$ - 2.350 $A_{645}$
Cholorophyll b = 18.61 $A_{645}$ - 3.960 $A_{662}$
Total carotene = 1000 $A_{470}$ - 2.270 Ca - 81.4 Cb/227
[Ca = Chlorophyll a, Cb = Chlorophyll b]

All the readings were taken in triplicate and their mean (± S.D.) was calculated accordingly.

**Statistical Analysis:** Two way ANOVA was also used to obtain morphological and physiological variability amongst the collected germplasm. Correlation was used to analyze relationship among different morphological and physiological characters of seeds and seedlings of *Nigella sativa*.

## Results and Discussion

**Morphological characterization:** The microscopic study of seed morphology revealed a number of variable traits. Seeds of *Nigella sativa* were found to be characteristically heart shaped with one end pointed while other end bulging as seen in figure-1. In the collected samples, seeds of Delhi has blunt bulging distal with pointed proximal end, while seeds of Uttar Pradesh has round bulging distal end. The seeds of Sikkim has typically straight bulging distal end and seeds of Kerala has narrow bulging distal end. Thus, morphologically seeds can be divided in two sub groups, blunt bulging (Delhi, Karnataka and Sikkim) and round bulging (in all other cases). Such morphological features may be used to identify germplasm tentatively as the same have been found significant for the study of seeds and their properties[17,18].

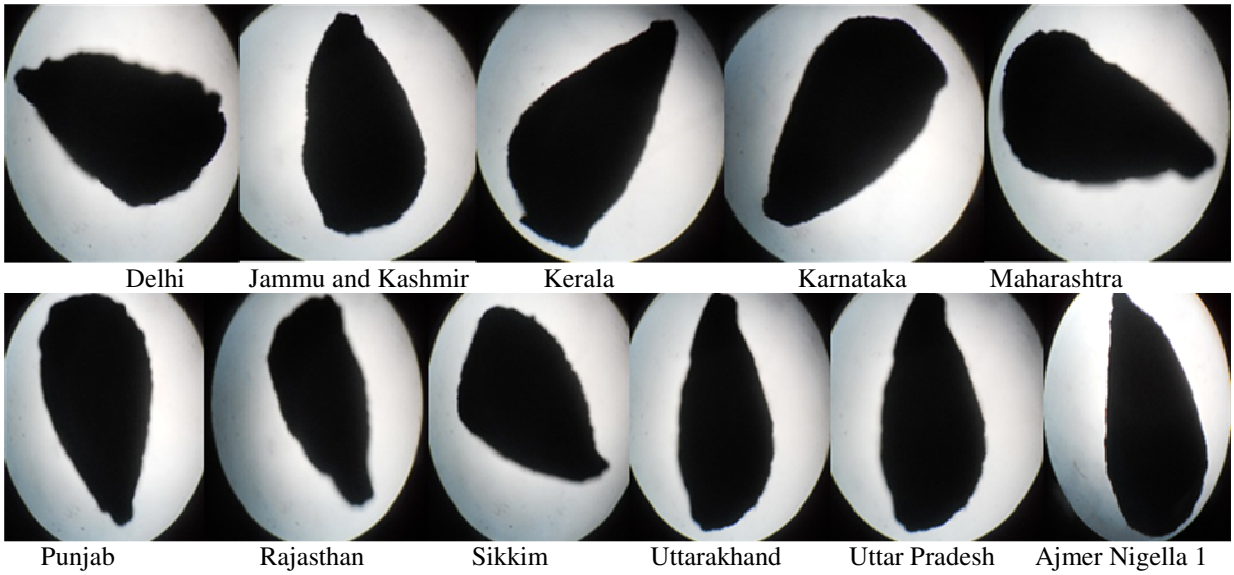


**Figure-1**
**Microscopic examination for shapes of *Nigella sativa* seeds**

In the seeds of *Nigella sativa* presence of a rough surface with edgy sides was a common feature noted in all seeds, although seed coat color of *Nigella sativa* exhibited variability. This variability in seed coat color, if consistent in further studies on similar germplasm, could be an important commercial trait. Similarly, importance of seed coat color has been studied and found to have relevance in other plants as well. The relationship between seed coat color and seed quality in watermelon Crimson sweet has been studied and found important[19]. No reference literature was found available for assigning the color (name/ range) to *Nigella sativa* seed coat. This is the first attempt to diversify the germplasm on the basis of seed coat color of *Nigella sativa* cultivars in India.

Average weight of 1000 seeds of *Nigella sativa* was taken, results are given in table-1. Highest weight was observed with seeds of Kerala (2.40 grams), while lowest weight was found in seeds of Uttarakhand (2.16 grams). On the other hand, when average size of seeds was taken, largest size was observed in seeds of Punjab (3.01mm in length and 2.0mm in width), while smallest size was found in seeds of Uttarakhand (2.83mm in length and 1.6mm in width). A similar study on shape, size and weight of weed seeds has been found valuable for weed recognition and organization[20]. Similar study has been done in *Indigofera* (Fabaceae) where dissimilarity was found in seed surfaces, seed shapes and epidermal cells within traditional species[21]. The results, of *Nigella sativa* cultivar in India, may thus, holds good for the morphological characterization of existing germplasm.

The study also revealed variation in the average root and shoot length of Nigella sativa seedlings, observed after 10 days of germination as given in table-1. It revealed minima in seeds of Rajasthan (root length with 1.6 cm and shoot length of 3.5 cm), while seeds of Karnataka exhibited maxima (6.1 cm shoot length and 3.3 cm root length). Similar study in cotton plant has been done, where significant variability was observed between genotypes which helped revealing the germplasm diversity based on physiological parameters[22]. Variations in the morphology of seedling as drought-induced changes in shoot and root growth of young cotton plants have also been reported[23]. When average leaf area was compared, Delhi seedlings exhibited maximum leaf area with 12.06 $mm^2$ and Punjab with minimum leaf area of 7.83$mm^2$. This feature finds no reference available in literature in this respect.

**Physiological characterization:** In Nigella sativa fresh weight and dry weight ratio of the seedlings was calculated by taking the average weight of ten randomly taken seedlings. The results are given in table-2. Maximum fresh weight was observed in case of seedlings from Jammu and Kashmir (0.246 g) and minimum (0.217 g) with that of Sikkim. Similarly, the maximum dry weight was observed with seedlings from Kerala (0.019 g) while minimum was observed with seedlings from Uttarakhand (0.010 g). The ratio of fresh weight/ dry weight was found maximum in case of Uttarakhand (24.10±2.029) while minimum was observed with seedlings from Kerala (12.47±2.380). These features may be important parameters, which influence the seed survival, germination potential and plant vigor/ resistance. Fresh and dry weight changes and germination ability of natural or prematurly desiccated developing wheat seeds have been studied, where it has been established that the lesser initial moisture content of desiccated seeds corresponds to higher amounts of water absorbed in the course of germination[24].

**Table-1**
**Morphological variation in *Nigella sativa* cultivar in India**

| | | Analysis of Seeds | | | Analysis of Seedlings | | |
|---|---|---|---|---|---|---|---|
| **Location** | **Seed Coat Color** | **Wt. of 1000 Seeds (Grams) ± S.D** | **Length of seeds (mm) ± S.D.** | **Width of seeds (mm) ± S.D.** | **Shoot length (cm) ± S.D.** | **Root length (cm) ± S.D.** | **Leaf area ($mm^2$) ± S.D.** |
| Delhi | Jet black | 2.26±0.020 | 2.85±0.037 | 1.6±0.081 | 3.9±0.739 | 2.5±0.408 | 12.06±0.170 |
| Jammu & Kashmir | Onxy black | 2.39±0.012 | 2.97±0.021 | 1.5±0.163 | 5.9±0.637 | 2.6±0.081 | 9.45±0.372 |
| Kerala | Olive black | 2.40±0.016 | 2.91±0.008 | 1.7±0.081 | 5.1±0.627 | 2.7±0.559 | 9.22±0.275 |
| Karnataka | Olive black | 2.28±0.014 | 2.9±0.026 | 1.9±0.057 | 6.1±0.852 | 3.3±0.163 | 10.32±0.551 |
| Maharashtra | Eerie black | 2.24±0.017 | 2.89±0.02 | 1.8±0.057 | 5.8±0.624 | 3.4±0.711 | 8.58±0.371 |
| Punjab | Onxy black | 2.32±0.021 | 3.01±0.021 | 2.0±0.081 | 6.1±1.576 | 3.2±0.535 | 7.83±0.139 |
| Rajasthan | Jet black | 2.23±0.022 | 2.92±0.032 | 1.9±0.057 | 3.5±0.544 | 1.6±0.294 | 10.44±0.553 |
| Sikkim | Davy's gray | 2.26±0.016 | 2.87±0.014 | 1.6±0.081 | 4.8±0.927 | 2.3±0.288 | 8.51±0.408 |
| Uttarakhand | Olive black | 2.16±0.024 | 2.83±0.021 | 1.6±0.081 | 5.0±0.408 | 2.8±0.238 | 9.54±0.401 |
| Uttar Pradesh | Olive black | 2.28±0.012 | 2.86±0.016 | 1.7±0.057 | 5.1±0.852 | 2.7±0.525 | 10.87±0.265 |
| Ajmer nigella (AN1) {reference} | Jet black | 2.40±0.020 | 2.93±0.057 | 1.3±0.288 | 5.6±0.208 | 3.1±0.763 | 8.16±1.258 |

Values are expressed as mean ±S.D.(n = 3)

**Table-2**
**Physiological variation in *Nigella sativa* germplasm of India**

| Location | Fresh Weight (grams) | Dry Weight (grams) | Water content (grams) | Fresh / Dry Weight ±S.D. | Chlorophyll A µg /gfw±S.D. | Chlorophyll B µg /gfw±S.D. | Total Carotene µg /gfw±S.D. |
|---|---|---|---|---|---|---|---|
| Delhi | 0.228 | 0.013 | 0.215 | 17.53±1.665 | 6.58±0.665 | 3.84±0.96 | 918.44±69.06 |
| Jammu & Kashmir | 0.246 | 0.014 | 0.232 | 17.57±2.113 | 7.54±0.575 | 4.61±1.04 | 803.21±83.93 |
| Kerala | 0.237 | 0.019 | 0.218 | 12.47±2.380 | 7.97±0.254 | 3.4±0.36 | 1001.18±97.91 |
| Karnataka | 0.222 | 0.012 | 0.210 | 18.50±1.149 | 7.65±0.620 | 3.5±0.62 | 1008.24±145.85 |
| Maharashtra | 0.238 | 0.013 | 0.225 | 18.30±1.439 | 8.03±0.300 | 3.56±0.74 | 810.15±83.09 |
| Punjab | 0.231 | 0.018 | 0.213 | 12.83±1.420 | 7.48±0.575 | 3.71±0.84 | 928.26±65.84 |
| Rajasthan | 0.223 | 0.015 | 0.208 | 14.86±1.075 | 7.18±0.225 | 4.35±1.49 | 799.80±56.52 |
| Sikkim | 0.217 | 0.012 | 0.205 | 18.08±1.871 | 8.21±0.495 | 3.28±0.20 | 868.65±52.58 |
| Uttarakhand | 0.241 | 0.010 | 0.231 | 24.10±2.029 | 7.22±0.927 | 4.32±1.56 | 988.29±101.36 |
| Uttar Pradesh | 0.230 | 0.013 | 0.217 | 17.69±3.224 | 7.67±0.556 | 4.51±1.15 | 1045.19±134.88 |
| Ajmer nigella (AN1) {reference} | 0.258 | 0.015 | 0.243 | 17.20±1.989 | 7.46±0.556 | 4.58±1.24 | 882.56±127.69 |

Values are expressed as mean ±S.D.(n = 3)

Estimation of cholophyll a, b and total carotene in Nigella sativa was done and results are presented in table 2. Chlorophyll a content in all the samples was studied and was observed that Sikkim has highest content (8.21±0.495 µg /gfw) and seedlings from Delhi exhibited least chlorophyll a content (6.58±0.665 µg /gfw). Moreover, chlorophyll A level in other samples was very close to each other. However, no significant difference in chlorophyll B content was observed among the gemplasms. When total carotene content was determined, the maximum content was observed in seedlings from Uttar Pradesh with 1045.19 µg /gfw and lowest in Rajasthan sample with 799. 80 µg /gfw. The correlation was calculated between weight of seed and shoot length (0.40727), which was found to be fairly significant. While in Plant length and fresh weight (0.327104) was calculated and plant length to water content (0.324575) was observed. When correlation was established between seed weight and carotene content (-0.04601) negative correlation was observed. The results show significant correlation among the morphological and physiological traits in Nigella sativa. Chlorophyll a, b and total carotene concentrations may be correlated to the photosynthetic potential of a plant and give some sign of the physiological status of the plant[25, 26]. However when two way ANOVA was applied, it also supported significant variation amongst the samples of Nigella sativa cultivar in India (with P- value < 0.05). The results showed morphological variability amongst seeds parameters (P-value 0.02), and seedling parameters (P-value 0.0007). When seeds and seedlings parameters were analyzed together, significant variability among the collected germplasm was observed (P-value 0.01). Physiological variation was also noted when analyzed for fresh weight, dry weight and water content of seedlings (P-value 0.004).

Since, morpho-physiological aspects change with change in different environmental conditions, and seem to be the outcome of responses to abiotic factors such as soil moisture[27], air temperature[28] and atmospheric $CO_2$ concentrations[29]. It was established that day length affects seed coat structure, while edaphic factors are accountable for the dissimilarity[30]. Both the factor may be participating in introducing variability in Nigella sativa which showed differences in morphology and physiological parameters in the collected germplasm. The results varied may be due to the environmental factors under which the seeds were produced in these states of India. Morphological and physiological parameters, studied here, may otherwise be of use in identification of germplasm, if genetically controlled. However, the genotypic study revealing and confirming variability still remains to be done.

## Conclusion

The present study explores morphological and physiological parameters which may be used to distinguish the cultivars based on features of seed and seedling. The seeds display diversity in shape, dimensions, weight and seed coat color. Seedling also exhibit variation in morphological and physiological parameters including color of fresh weight/ dry weight ratio, and relative size of shoot and root, chlorophyll and carotene contents. Morphological and physiological study of seeds and seedlings revealed considerable diversity among different cultivars of same species of Nigella sativa. This kind of study with more diverse germplasm including reference material may help to establish existence of diversity among Indian germplasm and would help to characterize them for further study at genotypic and molecular level.

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