Cytomorphological Studies and Seed Protein Characterization of *Nigella sativa* L. and *Nigella damascena* L.

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Summary Cytomorphological studies including scanning electron microscopy of the seed surfaces and seed protein characterization have been performed to ascertain interrelationship between 2 species of *Nigella* (Ranunculaceae) namely, *Nigella sativa* L. (black cumin: spice of commerce) and *Nigella damascena* L. (love-in-a-mist: ornamental) and the results obtained have been discussed. Further, in this investigation it has also been noted that a line of *N. damascena* obtained from Royal Botanic Garden, Kew, London possessed unique ornamental phenotype and yielded good number of viable seeds per plant which were with high protein content.

Key words *Nigella sativa*, *Nigella damascena*, Cytomorphology, SEM, SDS-PAGE.

Black cumin (*Nigella sativa* L., $2n=12$), a member of the family Ranunculaceae is widely cultivated for spice yielding property and therapeutic uses (Pruthi 1998) including anticancerous activity (Worthen *et al.* 1998). Improvement in *N. sativa* has been attempted through induced mutagenesis and colchiploidy and some desirable 'plant types' have been screened (Datta and Biswas 1985, 1986a, b, Rang and Datta 2001, Datta and Rang 2001, Datta and Saha 2001, Saha and Datta 2002a–c). However, improvement in yield and quality in *N. sativa* can also be made by exploring gene pool from related species. *Nigella damascena* L. (commonly known as love-in-a-mist, $2n=12$) possessing unique floral characteristics and bushy habit with enhanced number of branches and capsules and with higher seed protein content (Chakraborty and Chakraborty 1964), is one such species. Transfer of the better traits from *N. damascena* to *N. sativa* is most desirable but such attempts have not been made; although, such crosses might be successful as both species have equal chromosome number. Present investigation deals with cytomorphological studies including scanning electron microscopy of seed surfaces of *N. sativa* and *N. damascena* to ascertain interrelationship between the species for species hybridization. Further, electrophoretic pattern of seed protein has also been observed to decipher the similarities and differences between the species.

Materials and methods

Seed samples of *Nigella sativa* L. (cultivated variety-Persian jewels) and *N. damascena* (cultivated variety-Miss jekyll blue) obtained from 'Sutton and Sons', Kolkata and New Delhi respectively and *N. damascena* (accession no. 0016287) collected from Royal Botanic Garden, Kew, London were used in the present investigation.

Morphological studies

Plants from 3 seed stocks were raised (sowing of seeds on same date-15th November) in the experimental field of Kalyani University during the year 1998–1999 and to avoid any phenotypic

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variations due to environment, the selfed lines of those plants were grown in 2 subsequent generations (1999–2000, 2000–2001) in randomized block design with 3 replications (2000–2001). Each plot (4 m×2 m) consisted of 4 rows with spacing of 40 cm between rows and 30 cm between plants. Twenty five (*N. damascena*-Sutton) to 32 (*N. sativa* and *N. damascena*-Kew) randomly selected plants from 3 replications were estimated for different qualitative and quantitative morphological attributes from seedling stage to maturity (Table 1). Flowers of identical maturity were used for colour identification and the colours were determined from Horticultural colour chart I and II (1968). Pigmentation on the suture of the fruits in *N. damascena* plant types was also identified. Seed viability of the plant types was tested by dipping half seeds (50 half seeds from each plant type) in 1% aqueous solution of tetrazolium chloride for 24 h and embryos of seed stained red were considered viable.

Seed surface study following Scanning Electron Microscopy: For SEM studies, dry and clear seeds from each plant type (mother stock in each case) were fixed on a stud with double sided tape. They were coated with gold palladium using SEM coating unit ES 100 (Polorom Equipment Ltd.) and observed under SEM test (Model-JSM 5200 Tokyo, Japan) at 25 kV accelerating voltage at USIC, Jadavpur University, Kolkata. The photographs were taken at different magnifications.

Cytological studies

Karyotype analysis was made (seeds of original mother stock have been used) in the plant types following Datta and Biswas (1983). For meiotic studies, flower buds from the plant types (plants raised from original mother stock) of proper sizes were fixed in acetic alcohol (1 : 3) for 24 h and preserved in 70% alcohol. Anthers were squashed in 1% acetocarmine stain. Pollen fertility was assessed from temporary smears of pollen grains of freshly open flowers in drop of 1% acetocarmine. Stained pollen grains were considered fertile.

Seed protein characterization

Seed protein content of the 3 plant types (seeds of original mother stocks have been used) was estimated following Lowry *et al.* (1951). To study seed protein polymorphism, SDS-PAGE (10.0% separating gel and 4.5% stacking gel) was carried out in 2 dimensional gel following Laemmli (1970) in a vertical gel system (Biotech). Protein was loaded in each lane (30 µm) and the gel was stained in Coomassie Brilliant blue-R250 for overnight. Relative mobility (R_m) of the protein bands was determined after destaining and fixation of the gel.

Chlorophyll estimation

Quantitative assessment of chlorophyll 'a' and chlorophyll 'b' was made from leaf tissues (of same maturity) of the plant types (plants raised from seeds of mother stocks) following the method of Arnon (1949).

Estimation of essential oil

Essential oil was quantitatively assessed from seeds of the plant types (due to paucity of seeds of original mother stocks, seeds obtained from selfed lines in 2000–2001 were used; 3 replicas in each case and 1 g seed was used for each set of experiment) following steam distillation process as mentioned by Sengupta (2001).

Results and discussion

Morphological characteristics

On examination of the qualitative morphological characteristics (Table 1) it can be inferred that *N. sativa* having an erect habit (Fig. 1) varied conspicuously from the plant types of *N. damas-*

Table 1. Qualitative and quantitative characters in 3 plant types of *Nigella*

Sl. No.	Attributes	<i>N. sativa</i>	<i>N. damascena</i> (Sutton)	<i>N. damascena</i> (Kew)
1	Habit	Erect	Semi prostrate, bushy	Prostrate at seedling stage but rosette in appearance at maturity
2	Plant height (cm)	(30.0–67.6) 52.18 ± 4.42	(29.5–54.0) 42.27 ± 1.56	(17.1–58.2) 43.35 ± 1.43
3	No. of primary branches/plant	(4–10) 7.00 ± 0.71	(4–11) 10.81 ± 0.82	(8–38) 22.03 ± 1.12
4	Leaf arrangement	Alternate, leaf phyllotaxy 1–2	Spiral, leaf phyllotaxy 1–4	Spiral, leaf phyllotaxy 1–4
5	Nature of pinnae	Broad, elongated	Broad, elongated	Narrow, thick
6	No. of pinnae/rachis	5–6	9–11	7–8
7	Chlorophyll content in leaves (mg/g of tissue): Chlorophyll 'a' Chlorophyll 'b' Total branches/plant	1.007 0.665 (6–48)	1.204 0.874 (9–75)	1.347 0.990 (119–140)
8	Flower size (cm ³)	22.50 ± 4.11	27.19 ± 3.63	71.10 ± 5.95
9	Flower colour	2.74 × 2.78	3.23 × 3.25	3.86 × 3.77
10	Flowers with involucre of bracts	French Blue 43/3	Gentian Blue 42	Sea Blue 0 43/1
11	Nature of peduncle	–	+	+
12	Nature of petaloid sepals	Long, erect	Long, decumbent	Long, decumbent
13	No. of whorls of petaloid sepals	Broad, ovate	Narrow, elongated	Broad, elongated
14	No. of petaloid sepals	1	3–4	1
15	No. of petaloid sepals	4 to 6	34–40	5
16	Flower fertility (%)	89.89	33.93	63.82

Table 1. Continued

Sl. No.	Attributes	<i>N. sativa</i>	<i>N. damascena</i> (Sutton)	<i>N. damascena</i> (Kew)
17	No. of microsporophyll/flower	(32-66) 43.6±2.7	(16-34) 25.7±2.1	(47-69) 56.4±2.0
18	No. of capsule/plant	(5-45) 20.00±3.71	(1-20) 9.52±1.92	(11-99) 44.67±3.81
19	Capsule fertility (%)	94.50	15.56	52.61
20	Nature of capsules	Elongated to roundish without bracts	Globular-oblong, inflated with bracts	Globular-oblong, inflated with bracts
21	Pigmentation on capsules	—	Plum purple (934) on suture	Plum purple (934) on suture
22	Length of capsules (cm)	(0.4-1.5) 1.03±0.13	(0.9-2.0) 1.42±0.03	(1.2-2.5) 1.78±0.03
23	Seta/capsule	(4-8) 5.10±0.10	(5-9) 5.30±0.11	(5-6) 5.03±0.01
24	Filled seeds/capsule	(6-100) 59.29±3.20	(0-72) 6.14±1.53	(0-103) 46.59±2.16
25	Total seeds/plant	(238-2160) 935.20±177.94	(0-72) 17.66±5.75	(103-2046) 870.63±189.06
26	Seed weight/plant (g)	(0.5-5.0) 1.91±0.38	(0.0-0.07) 0.01±0.01	(0.1-3.42) 1.03±0.19
27	Seed protein content mg/g of tissue	14.346	21.207	22.684
28	Duration of vegetative phase (days)	64-80	145-160	130-150
29	Duration of flowering phase (days)	45-55	28-33	41-60
30	Germination frequency (%)	70-88	36-40	70-80
31	Survivality (%)	100	10-16	100
32	Seed viability (%)	80-96	40-46	86-94
33	Essential oil content (%)	1.2-1.5	0.9-1.1	1.2-1.3

Values in parenthesis indicated range.

Figs. 1–3. 1) Erect habit in *N. sativa*. 2) Semi-bushy nature of *N. damascena*-Sutton. 3) *N. damascena*-Kew showing rosette habit with long decumbent floral shoots.

cena; while, *N. damascena*-Sutton and *N. damascena*-Kew plants differed markedly between themselves in relation to habit (Figs. 2, 3), nature of pinnae, floral characteristics, survivality and seed viability. Similarly, quantitative morphological attributes (Table 1) were found to vary significantly among the plant types ($p < 0.001$ in all cases excepting capsule length which showed variation at 0.05 probability level; however, seta per capsule showed non-significant variations) and between the plant types of *N. damascena* ($p < 0.001$ in all cases excepting plant height which demonstrated non-significant variation) as evidenced from χ^2 test of heterogeneity and t -test respectively.

Having enhanced flower and capsule sterilities, the *N. damascena*-Sutton plants were mostly seedless and rarely yielded few good seeds per plant and the seeds were poor in viability (Table 1). Further, the plants of *N. damascena*-Sutton showed sign of drying up at the flowering stage (April–May). On the contrary, *N. damascena*-Kew plants produced enhanced number of flowers, capsules and seeds per plants and manifested 100% survivality (Table 1), thereby suggesting their good adaptability in present condition. These plants possessed unique rosette phenotypes with thick dark green pinnae of leaves and large flowers and had single whorl of petaloid sepals in flowers like *N. sativa* (Figs. 1, 3, $\Rightarrow$). Persistent presence of 3 to 4 whorls of petaloid sepals in flowers of

Table 2. SEM analysis of seed surfaces of three plant types

Sl. No.	Attributes	<i>N. sativa</i>	<i>N. damascena</i> (Sutton)	<i>N. damascena</i> (Kew)
1	Seed shape	Ovate, tetragonal, angles sharp, acute, more tapering at the end (Fig. 4)	Ovate, triangular, angles sharp, acute, shallow tapering at the end (Fig. 8)	Ovate, triangular, angles sharp, acute, tapering at one end (Fig. 12)
2	Seed size (cm ²)	2.33±0.1×1.14±0.02	2.30±0.1×1.64±0.05	1.95±0.05×1.24±0.04
3	Seed surface	Reticulation marks present, reticulation more prominently raised, reticulation pentagonal to polygonal, ovoid or irregular in outline (Fig. 5)	Reticulation marks present, reticulation mediumly raised, reticulation tetra- and pentagonal, ovoid (Fig. 9)	Reticulation marks present, reticulation prominently raised, reticulation tetra-, penta-, and polygonal (Fig. 13)
3.1	Nature of reticulate rows	Reticulate rows (Fig. 5—⇨) consisting of smaller tuberculate raised cell (Fig. 7—⇨); cells either uni- or multiseriate or in aggregation along the corners or junctions	Reticulate rows (Fig. 9—⇨) consisting of tuberculate raised cell; cells 2 to 3 seriate; uniformly arranged never aggregated	Reticulate rows (Fig. 13—⇨) consisting of smaller tuberculate raised cell; commonly of 4 to 5 rows, no aggregation along junction or corner
3.2	Cells of reticulate lines	Showed shrinkage structure	Undifferentiated mass, coated dermal appearance (Fig. 10—⇨)	Distinct, tuberculate, long paraphysis (Fig. 15—⇨) or eliphore like structure more often with coated dermal appearance or with the appearance of semi-coralloid structure (Fig. 15—⇨)
3.3	Nature of bounded area	Variable number of cells (2–5); each cell comparatively larger, penta-, hexa-, polygonal or rounded in outline (Fig. 5—⦿)	9 to many cells; cells comparatively medium in size, polygonal to rounded in outline. Less often penta- to hexagonal (Fig. 9—⦿)	More than 20 cells; comparatively smaller; cells penta- to hexagonal in outline (Fig. 13—⦿)
3.4	Lumen floor	Depressed or shallow glabrous (Fig. 6—⇨)	Acutely angled, often pyramidal terminated into acute tips, surface glabrous (Fig. 10—⇨)	Obtusely rounded, rarely with obconical outline (Fig. 14—⇨)

N. damascena-Sutton (Fig. 2, ⇨), provided ornamentation; however, finely dissected involucre of bracts were present in flowers and fruits of both *N. damascena*-Sutton and *N. damascena*-Kew plants. As compared to *N. sativa*, vegetative phase has been prolonged in *N. damascena* plant types and the duration of flowering phase and flowering time (*N. sativa*—late January to 3rd week of March, harvest—end of March; *N. damascena*-Sutton—1st week of April to 1st week of May, harvest—mid of May; *N. damascena*-Kew—2nd week of March to 1st week of May, harvest—mid of May) have been noted to vary among themselves (Table 1).

Seed surface analysis following SEM: Seeds of *N. damascena*-Sutton were broader, while, seeds of *N. damascena*-Kew were smaller in sizes (Table 2). SEM study of seed surfaces (Figs. 4–15) revealed distinct variations and similarities among the plant types in relation to seed shape, reticulation marks, cells of reticulation, bounded area and lumen floor (Table 2). Marked variations have also been noted between seed surfaces of *N. damascena*-Sutton and *N. damascena*-Kew.

Cytological studies

Mitotic chromosome analysis performed in the plant types revealed $2n=12$ chromosomes (Figs. 16–18) in agreement to previous reports (Gregory 1941, Bhattyacharya 1958, Kumar and

Figs. 4–15. Scanning Electron Microscopy of seed surfaces. 4–7) *N. sativa*. 8–11) *N. damascena*-Sutton. 12–15) *N. damascena*-Kew.

Nizam 1976, Datta and Biswas 1983, Saha and Datta 2002c) and the chromosomes were of 4 types in all cases (Table 3) based on chromosome length, nature of primary constriction and presence or absence of secondary constrictions. The chromosomes in 3 plant types formed graded karyotypes and the karyotypes were symmetric in nature (TF%–41.38 to 44.18). The somatic chromosome complements of *N. damascena* (Sutton and Kew samples) possessed 2 pairs of chromosomes with secondary constrictions; while, *N. sativa* had secondary constrictions in 1 pair of chromosomes. Haploid chromatin length has been noted to be highest in *N. damascena*-Kew among the plant types (Table 3).

The chromosomes at metaphase I (Table 3) mostly formed bivalents (5.79 to 5.95) and rarely univalents (0.10 to 0.40) per cell. Rod bivalent ($0.20 < p < 0.30$) demonstrated random distribution, while ring bivalent per cell ($p < 0.01$) and chiasma per nucleus ($p < 0.02$) were non-random among the plant types as evidenced from χ^2 test of heterogeneity. About 22.98% of the AI meiocytes of *N. damascena*-Sutton (Table 3) demonstrated chromosomal aberrations in the form of dicentric chromatid bridge with an accompanying fragment, laggards (1–4), tripolar organisation of chromosomes and unequal separation of chromosomes; while, in other 2 plant types occasional laggards (1–2) were only observed. Average pollen fertility was high in the plant types (81.61–98.06%).

Figs. 16–18. Somatic cells of *N. sativa* (16), *N. damascena*-Sutton (17) and *N. damascena*-Kew (18) showing $2n=12$ chromosomes. Chromosomes with secondary constrictions ($\Rightarrow$) have been marked.

Table 3. Chromosomal attributes in *N. sativa* and *N. damascena* plant types

Attributes	<i>N. sativa</i>	<i>N. damascena</i> (Sutton)	<i>N. damascena</i> (Kew)
Mitotic parameters:			
Chromosome no.	12	12	12
Chromosome types	2A+2B+6C+2D	2A+4B+4C+2D	2A+4B+4C+2D
Range of chromosome length (μm)	6.64–19.13	5.29–17.66	6.0–18.59
No. of chromosomes with sec. const.	2	4	4
Haploid chromatin length (μm)	85.6 ± 3.3	71.5 ± 1.7	94.6 ± 3.1
TF%	41.38	44.18	42.32
S%	34.71	29.95	32.30
Karyotype formula	$2A_m + 2B_m^{sc} + 6C_m + 2D_t$	$2A_m + 4B_m^{sc} + 4C_m + 2D_t$	$2A_m + 4B_m^{sc} + 4C_m + 2D_t$
Meiotic parameters:			
Chromosome association/cell at MI	5.95 II+0.10 I	5.79 II+0.40 I	5.83 II+0.33 I
Ring bivalent/cell	3.18 ± 0.35	2.98 ± 0.26	2.75 ± 0.20
Rod bivalent/cell	2.77 ± 0.28	2.86 ± 0.27	3.07 ± 0.22
Chiasma/nucleus	9.34 ± 0.28	8.94 ± 0.22	8.35 ± 0.25
PMC scored at MI	175	150	148
Equal 6/6 AI sep. (%)	94.49	77.02	98.37
Total cell analyzed at MI	394	148	184
Pollen fertility (%)	98.06	88.01	81.61
No. of pollen grains studied	1174	344	1383

m=median, sc=secondary constriction, t=telocentric.

Seed protein characterization

Seed protein content (Table 1) was maximum in *N. damascena*-Kew (22.684 mg/g of tissue) and minimum in *N. sativa* (14.346 mg/g of tissue). SDS-PAGE showed differences in the number of bands (Fig. 19), although band width and intensity was similar among the plant types (Table 4). *N. damascena*-Kew exhibited 16 bands while, *N. damascena*-Sutton and *N. sativa* demonstrated 12 bands. Bands '2' and '6' have been found to be specific in *N. damascena*-Kew, while, band '4', '12' and '16' were common in *N. damascena* plant types. Band '7' and '17' have been noted in both *N. sativa* and *N. damascena*-Kew but band no. '3' was only observed in *N. sativa*. Results suggested that electrophoretic characterization of seed protein may be used as an additional parameter to supplement morphological and cytological data in understanding genetic interrelationship among the plant types.

Present study demonstrated gross similarities and differences among/and between the plant types. *N. damascena*-Kew possessed some unique traits specific to themselves apart from *N. damascena* characteristics; however, few traits were found to be similar to *N. sativa*. Results also indicated that *N. damascena*-Sutton plants offered lesser scope of their utilization as germplasm source for species hybridization with *N. sativa*. On the contrary, *N. damascena*-Kew plants having an ornamental phenotype and yielding high number of seeds per plant with good viability and the seeds possessing good amount of protein and essen-

Fig. 19. SDS-PAGE electrophoretic pattern of seed protein from *N. sativa* (A), *N. damascena*-Sutton (B) and *N. damascena*-Kew (C).

Table 4. SDS-PAGE banding pattern of seed protein in three plant types

Band No.	R _m value	<i>N. sativa</i>	<i>N. damascena</i> (Sutton)	<i>N. damascena</i> (Kew)	Band intensity	Band width
1	0.03	+	+	+	I	IM
2	0.11	—	—	+	I	B
3	0.29	+	—	—	I	IM
4	0.35	—	+	+	I	IM
5	0.43	+	+	+	F	N
6	0.47	—	—	+	F	N
7	0.53	+	—	+	I	N to B
8	0.59	+	+	+	I	N
9	0.65	+	+	+	I	N
10	0.67	+	+	+	I	IM
11	0.74	+	+	+	I	IM
12	0.77	—	+	+	I	IM
13	0.81	+	+	+	I	N
14	0.84	+	+	+	I	IM
15	0.89	+	+	+	I	IM
16	0.95	—	+	+	I	IM
17	0.98	+	—	+	F	N

F=Faint, I=Intense, N=Narrow, B=Broad, IM=Intermediate.

tial oil seems to be better suited in present condition and seeds of such a plant type may serve as important genetic stock for further exploration and exploitation in the field of cytogenetics; however, duration of cropping period has to be synchronized with *N. sativa* to offer possibility of interspecific hybridization.

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