MORPHOLOGICAL AND CYTOLOGICAL STUDIES IN *NIGELLA SATIVA* L. AND *N. DAMASCENA* L. (RANUNCULACEAE)

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Abstract: *Nigella sativa* L. (black cumin; potential herb with immense therapeutic uses apart from its spice yielding property; cultivated variety – *Persian Jewels*) and *Nigella damascena* L. (commonly known as 'love-in-a-mist', grown in gardens throughout temperate region of the world; cultivated variety – *Miss Jekyll blue* obtained from Sutton and Sons', Kolkata and an accession 0016287 obtained from Royal Botanical Garden, Kew, London) members of the family Ranunculaceae were grown in the Experimental field plots of Department of Botany, Kalyani University (Nadia, West Bengal plains, latitude 22°50′ to 24°11′ N, longitude 88°09′ to 88°48′ E, elevation 48 ft. above sea level, sandy loamy soil) for three consecutive years as rabi crop. The plant types were described and Kew accession was found to be unique and better adaptive than that of Sutton samples of *N. damascena*. Morphometric (plant height, primary and total branches/plant, capsule/plant, capsule length, seta/capsule, filled seeds/capsule, seed weight/plant as well as capsule and flower sterilities) and meiotic (mean chromosome association/cell at metaphase I, bivalent configurations, chiasma/nucleus, anaphase I segregation and pollen fertility) parameters were assessed in the plant types and statistical analysis (χ^2-test of heterogeneity and Student t-test) of the accumulated data revealed significant variations among/between plant types for most of the traits. Results indicated the possibility of efficient breeding between species/accessions for enhancing gene pool of *Nigella*.

Keywords: Efficient breeding, Meiosis, Morphometric traits, *Nigella damascena*, *Nigella sativa*.

INTRODUCTION

Nigella sative L. (common name – black cumin; Family: Ranunculaceae; annual herb with potential therapeutic uses and spice yielding property of commerce) is reported to be a model plant species for cytological and cytogenetical studies (Datta and Biswas 1985; Datta and Saha 2001; Datta and Rang 2001; Rang and Datta 2001; Saha and Datta 2002; Ghosh and Datta 2006). On the contrary, *N. damascena* L. (common name – 'love-in-a-mist'; Family: Ranunculaceae) is reported from India (Anonymous 1966) and grow in gardens throughout temperate regions of the world (vide Encyclopedia Britannica @ 1999-2000) and is a native of Mediterranean region. Efficient breeding endeavour between the species may widen the gene pool in *Nigella* for selection of desirable 'plant type(s)' of commercial interest. With the view to it, seeds stocks of *N. sativa* and *N. damascena* (two germplasm source used – one from Sutton and Sons', Kolkata, while the other from Kew garden, London) were grown (experimental field plots of Kalyani University, West Bengal plains, Nadia) for three consecutive years and described. Phenotypic variables and meiotic parameters were analysed in the plant types to assess significant variations, if any, for further exploration. The objective of studying meiosis is to understand the process (dealing with the course of microsporogenesis) which is pivotal in designing future programme on reproduction, fertility, genetics and plant breeding.

MATERIAL AND METHOD

Plant types: The seed samples of *Nigella sativa* L. (cultivated variety – *Persian Jewels*, obtained from Sutton and Sons', Kolkata; seed moisture content 7.5%; designated as NS) and *N. damascena* L. (cultivated variety – *Miss Jekyll blue*, obtained from Sutton and Sons', Kolkata, seed moisture content 13.33%, designated as NDS; seed samples obtained from Royal Botanical Garden, Kew, London – accession No. 0016287, designated as NDK) were grown in the Experimental field plots of Department of Botany, Kalyani University (West Bengal plains, Nadia, latitude 22°50′ to 24°11′ N, longitude 88°09′ to 88°48′ E, elevation 48 ft. above sea level, sandy loamy soil) as rabi crop during the months of November to mid-March for *N. sativa* and November to early May for *N. damascena* for three consecutive years. Seeds of the plant types were sown in lines keeping uniform distance between plants (25 cm apart) and lines (30 cm interval). Uniform cultural practices were maintained throughout and no fertilizer application was made

Morphometric analysis: Morphological attributes as presented in Table 1 were studied from 30 plants of *N. sativa*, 27 plants of *N. damascena* – Sutton and 30 plants of *N. damascena* – Kew (in all the plant types 9 to 10 plants were screened in each year randomly, excluding boarder plants; plants were scored from 3 different lines for each plant type for a year). Data recorded were pooled over the plants for each year and over the years for each plant type. Out of 27 NDS plants 17 were seedless. Flower (flowers not transformed to capsules were considered sterile) and capsule (capsules not yielding good filled seeds

were identified as sterile) sterilities, size and colour of the flowers (Horticultural Colour Chart I and II - 1968) and day to first flowering were also analyzed in the plant types.

Meiosis: Meiotic analysis was performed from 2 to 3 randomly selected plants of each plant type in each year. Suitable sized flower buds were fixed in acetic alcohol (1:3 v/v) for 24 hours, transferred to 70% alcohol and stored in a refrigerator for convenient use. Anther squash preparations were performed and PMCs were stained in 1% acetocarmine (Marks 1957). Fully stained pollen grains were considered fertile. Metaphase I (MI) configurations, chiasma per nucleus, anaphase I (AI) segregation and pollen fertility percentage were assessed and data were pooled over the plants and over the years for each plant type. Photomicrographs were taken from temporary squash preparations.

Statistical analysis: χ^2-test of heterogeneity (2 DF) was performed for phenotypic variables and meiotic parameters to assess significant variation among the three plant types under study. Test of significance (Student t-test) was also made for different morphological traits between *N. damascena* – Sutton and Kew samples to estimate significant variation between them.

FIGURE LEGENDS

Figure plate I (1-6) showing plant types, flowers and fruits of *Nigella* **(1)** N. *sativa* (NS); **(2)** *N. damascena* – Sutton (NDS); **(3)** *N. damascena* – Kew (NDK); **(4)** flowers – **(a)** NDK; **(b)** NDS; **(5)** *N. damascena* showing pigmentation on the suture of fruits; **(6)** fruits – **(a)** NS; **(b)** NDS; **(c)** NDK.

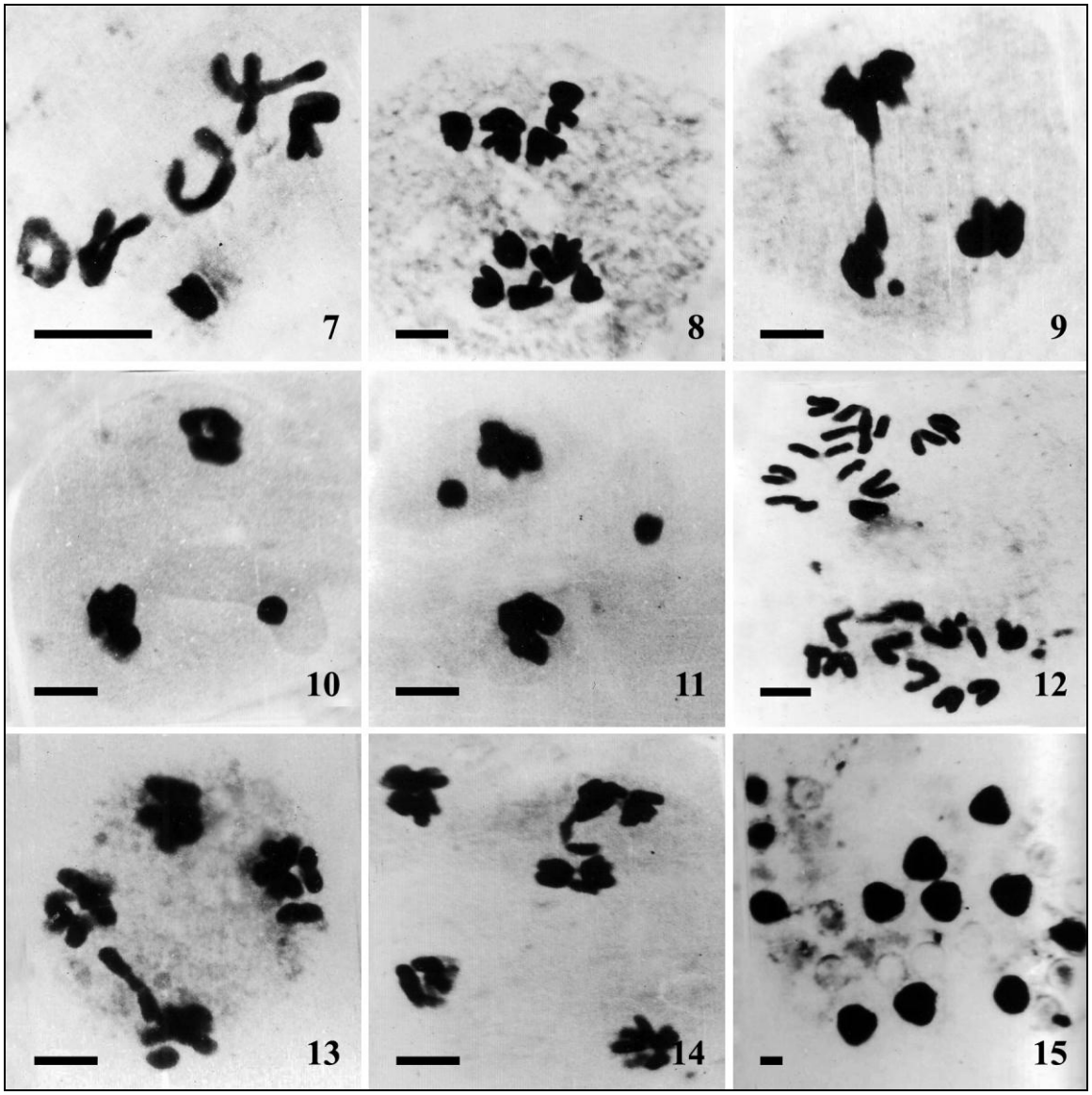

Figure plate II (7-15) showing meiotic configuration and pollen fertility in *Nigella* spp. **(7)** 6II at MI; **(8)** 6/6 separation of chromosomes at AI; **(9)** bridge with a fragment and tripolarity at AI; **(10-11)** AI with laggards; **(12)** irregular separation at MII; **(13-14)** bridge formation at AII; **(15)** stained and fertile pollen grains. Scale bar = 10 µm.

RESULTS AND DISCUSSION

N. satva is an erect (**Fig. 1**) annual herb with pale (French Blue 43/3) flowers (size: 2.74×2.78 cm) without involucres and borne solitary on long, erect peduncles. Flowers were with one whorl of petalloid sepals and they were broad and ovate in shape. Floral shoots were non-pigmented. Capsules were elongated to roundish in shape with no pigmentation (**Fig. 6a**) on the suture and capsules were without involucres of bracts. In NS, the vegetative phase (from germination to early flowering) was noted to be 64 to 80 days; while, the reproductive phase was assessed to be 40 to 55 days. The plants are harvested within 125 to 145 days from sowing. Viability of seeds ranged between 80.0% and 96.0% over the generations. *N. damascena* – Sutton is a bushy, semi-prostrate (**Fig. 2**), annual herb with large number (4 to 11, mean 10.81±0.82) of lax natured pigmented lateral branches; while NDK plants were prostrate at seedling stage but at maturity they were rosette in appearances due to the formation of short sized, compact and non-pigmented lateral branches (8 to 39, mean 22.03±1.12). In NDS, the flowers were large (3.23 cm×3.25 cm), gentian blue (42) with dense, finely cut involucres (**Fig. 4a**), 3 to 5 whorls of petalloid sepals (30 to 40) imparting ornamental value to the plants; sepals were narrow, elongated in nature; capsules were globular-oblong, inflated and with involucres of bract, plum purple (934) pigmentation on the suture. In NDK, the flowers were also large (3.86 cm×3.77 cm), sea blue (0 43/1) with finely cut involucre and with one whorl of petalloid sepals (**Fig. 4b**). Capsules in the plant types

were globular-oblong, inflated and with finely cut involucres and plum purple (934) pigmentation on the suture (**Figs. 5c, 6**). In both the plant types of *N. damascena* vegetative phase (NDS – 145 to 160 days; NDK – 130 to 150 days) was much prolonged than reproductive phase (NDS – 28 to 33 days; NDK – 41 to 60 days) consequently had delayed harvesting (NDS – 185 to 200 days; NDK – 193 to 200 days from sowing) compared to NS.

Morphometric and meiotic parameters studied in the plant types are presented in Table 1. Among the assessed morphological attributes capsule length was found to vary at 5% level, while all other traits (excepting seta/capsule) showed significant variation among the plant types at 0.001 probability level. Test of significance (t-test at 55 DF) conducted between NDS and NDK for the morphological parameters revealed that for excepting plant height ($t=0.37$, $p>0.05$) all other attributes (primary branches/plant: $t=8.07$, $p<0.001$; total branches/plant: $t=5.93$, $p<0.001$; capsule/plant: $t=7.69$, $p<0.001$; capsule length: $t=9.0$, $p<0.001$; seta/capsule: $t=14.45$, $p<0.001$; filled seeds/ capsule: $t=14.45$, $p<0.001$; seed weight/plant: $t=25.5$, $p<0.001$) varied significantly.

All plant types showed 2n=12 chromosomes uniformly in their meiocytes (**Figs. 7, 8**). Mean chromosome association per cell was 5.95II+0.10I in NS, 5.79II+0.41I in NDS and 5.83II+0.33I in NDK respectively. The bivalents formed ring and rod configurations. Frequency of ring bivalent per cell was higher than rod per cell in NS but rod/cell was enhanced than rings in NDK. Although bivalent frequency per cell was consistent ($p>0.05$), univalent/cell ($p<0.001$), chiasma /nucleus ($p<0.001$), ring ($p<0.05$) and rod ($p<0.05$) configurations of bivalents were non-randomly distributed among the plant types. AI segregation of chromosomes was nearly balanced (6/6) in NS (99.49%) and NDK (98.37%) but NDS (6/6 – 77.02%; rest of the cells showed unequal segregation of chromosomes, laggards, tripolarity and bridge formation with or without an accompanying fragment – **Figs. 9, 10, 11**) had disturbed separation to an extent. AII cells studied in the plant types were mostly cytologically balanced rare often irregular separation of chromosomes, laggards and bridges were observed in NDK (**Figs. 12, 13, 14**). Pollen fertility (**Fig. 15**) was recorded to be 98.06% in NS, 88.01% in NDS and 81.61% in NDK. Meiotic data therefore indicated that extremely low amount of seed yield in NDS is rather not cytological, it possibly may have genetic basis.

Table 1. Cytomorphological attributes in *N. sativa* and *N. damascena* plant types

Attributes	NS	NDS	NDK	P value of χ^2-test of heterogeneity at 2 DF
Morphological				
Plant height (cm)	52.18±4.42	42.27±1.56	43.35±1.43	<0.001
Primary branches/plant	7.00±0.71	10.81±0.82	22.03±1.12	<0.001
Total branches/plant	22.50±4.11	27.19±3.63	71.10±5.95	<0.001
No. of capsule/plant	20.00±3.71	9.52±1.92	44.67±3.81	<0.001
Capsule length (cm)	1.03±0.13	1.42±0.03	1.78±0.03	<0.05
Seta/capsule	5.10±0.10	5.30±0.11	5.03±0.01	<0.70
Filled seeds/capsule	59.29±3.20	6.14±1.53	46.59±2.16	<0.001
Seed weight/plant (gm)	1.91±0.38	0.01±0.01	1.03±0.19	<0.001
Flower sterility (%)	11.11	66.07	37.18	<0.001
Capsule sterility (%)	5.50	84.44	47.39	<0.001
Meiosis				
Bivalent/cell	5.95	5.59	5.83	>0.05
Univalent/cell	0.10	0.41	0.33	<0.001
Ring/cell	3.18±0.34	2.98±0.27	2.75±0.18	<0.05
Rod/cell	2.77±0.35	2.86±0.28	3.07±0.19	<0.05
Chiasma/nucleus	9.34±0.28	8.94±0.22	8.35±0.25	<0.001
Total cells scored at MI	175	150	148	-
Equal (6/6) AI separation (%)	99.49	77.02	98.37	-
Total AI cells scored	394	148	184	-
Pollen grains fertility (%)	98.06	88.01	31.61	-
Total pollen grains scored	1174	344	1383	-

CONCLUSION

Results of the present investigation suggested better adaptability of Kew-accession of *N. damascena* in Nadia than Suttons' variety. Cytomorphological variations encountered among the plant types may be explored through efficient breeding and selection. However, necessary approaches must be undertaken to shorten the growth period of *N. damascena* and make it near comparable to *N. sativa* for performing any breeding experiments.

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