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RESEARCHES CONCERNING THE BIOLOGY OF THE NIGELLA L.SPECIES (FAM.RANUNCULACEAE) FROM ROMANIA

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Abstract:
CORNEANU G.C., POPESCU M., SITORIS A., SILEANU V.D., POPESCU G., 1987, Researches concerning the biology of the Nigella L.species (Fam.Ranunculaceae) from Romania. Not.bot.hort.agrobot., Cluj.
Nigella L.species the utilization and biology of are presented. The anatomy, chromosome features, seed chemical composition, flower colour genetics and some somatic mutants of Nigella species from Romania are discussed.
Key words: Nigella spp., utilisation, biology, genetics.
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Nigella L. (Helleboreae, Ranunculaceae) comprise almost 20 annual species, found in the Mediterranean region (STRID, 197o). In recent decades, anatomical studies (METCALFE, CHALK, 1957; P... ŞIC..U, 1973 a.o.) and chromosome studies (STRID, 1965, 197o; MOU... ...N et al., 1966; GILOT-DELHALLE, 197o a.o.) on some species of Nigella genus were performed.

In Romania's flora, the Nigella genus is represented by three species (NYÁRÁDY, 1953), which were studied by FILIPESCU, 1969; CORNEANU, 1974, 1978 a.o.

In this study the chorology, the anatomy of stem and the leaves, the seed chemical composition, the chromosome features, as well as

some somatic mutants in Nigella species from Romania are presented.

The species of the genus were known since ancient times. N.sativa was described by HIPPOCRATES, CATO MAIOR, CAIUS PLINIUS SECUNDUS, PEDANIOS DIOSCORIDES; N.aristata (=N.arvensis L.ssp.aristata (Sibth. Sm/Nym.) by PEDANIOS DIOSCORIDES (FRAAS, 1845, LENZ, 1859).

PEDANIOS DIOSCORIDES from Anazarba in his famous work "Peri hiules iatriches" (De materia medica), 3rd book, chapter 62 and 84, as well as CAIUS PLINIUS SECUNDUS (II Century A.D.), in his monumental "Naturalis historia", 20th book, chapter 17, besides the description of the species of Nigella above mentioned, presents their use at length.

Nigella sativa, known by the Greeks under the name of git,melanthion, melanthium or melanperman, was used for broad preparation, treating for wounds caused by snake or scorpion bittings; tooth and headaches, mouth ulcerations, in wart, freckle and leprosy treatment, to increase woman's lactation, in diuresis in constipations treatment, to eliminate the belly worms, or to attenuate the difficulties of breathing.

With reference to its name, DIOSCORIDES notes: "the Roman call it [illegible] and probably [illegible], the name which is [illegible]". Hence it results that the plant was known since ancient times under the name of Nigella, Carl LINNAEUS taking over this name from the antiquity, in the same way as he proceed for a great number of species in his work "Species Plantarum", 1753 (VACZY, 1970).

DIOSCORIDES considers that N.aristata (named Agrion Melanthion or even melanthion) would represent the wild form of the N.sativa species. It was advised in the cure of the wounds in snake bites, to alleviate the vesicle or kidneys pains (seed tea) and to stop bleeding.

During the Middle Ages, Jacob THEODORUS TABERNAEMONTANUS in his "[illegible]" (1588) described six species of Nigella: N.sativa (the 1st described species), [illegible] (the 4th species), N.arvensis (the 5th species), [illegible] (the 6th species) and perhaps two cultivated forms of the N. [illegible] (2nd and 3rd species).

In the Herbarium of Péter MELIUS edited in Cluj in 1578 at least two of the Nigella species (N.sativa and N.arvensis) are mentioned, one as cultivated species and the other as a weed - both largely used as medicinal plants in about 17 recipies (19, 23).

The seeds of the Nigella species are aromatic and used as spice throughout the Levant. In Egypt, bread and cakes were strewn with seeds of Nigella to become more appetizing, and the women ascribed them the quality of rounding their body (GUIBOUT, PLANCHON, 1876).

MATERIAL AND METHOD

Biological material. N.arvensis L. ssp.arvensis, N.damascena L. ("single flower" and "double flower", "Miss Jekyll type) as well as N.sativa L. collected in Craiova were studied. The population of N.damascena "single flower" with a recessive homozygote gene for white flower colour (Utrecht, Holland) was analyzed for the genetic determinism of the flower colour and for the chemical composition of seeds.

Concordant with A.TERRACCIANO'S classification (1897, 1898), N.damascena belong to Erobates section, while N.arvensis and N.sativa belong to Erniella section, Nigellaria subsection (GILOMMELHALLE,1970).

Chorology. The chorology of the three species was studied by field observations herbarium and literature data.

Stem and leaf anatomy. Anatomical studies were performed after flowering on mature plants. The stem structure was studied on cross sections (median zone and internode II under the flower). The foliar limb structure was analyzed on cross sections in the median zone of the terminal branching of the leaves. The determination of the structural characteristics of the leaves (shape and size of epidermal cells, the type and density of stomata) were performed in the cauline leaves of the median zone of the stem. The obtained data (loo observations) were interpreted statistically.

Karyotype. Chromosome characteristics were performed on squash preparations, with Feulgen stain, from the root tops. For each species 12 metaphases were analyzed; the length of arms and satellites, the chromosome pairs which bear the satellites, the centromere index and the arms ratio.

Genetic determinism of flower colour. Seeds of Nigella arvensis, N.damascena "single flower" (Craiova population) and "double flower" ("Miss Jekyll" type) were exposed to X-ray radiation in the 3,5oo-6,5oo R range at a rate of 8oo-1,2oo R/min, and to fission neutrons in dosis of loo-6oo R. Somatic mutents were studied in descent. Genetic determinism of flower colour in N.damascena "single flower" was established

through hybridization.

Seed chemical composition. To establish the chemical composition, 5o g of fine ground Nigella seeds were extracted succesively with petroleum ether, ethylic ether, methilic alcohol and acidulated water (HCl 2 %). The identifying reactions for a large range of chemical constituents were caried out subsequently.

The free and combined fatty acids were separated and chromatographicaly identified from the saponification fraction of the lipophilic complex, utilizing the technics indicated by KAUFMANN and NITSCH (1952), using for analyses etheric solutions containing free and combined fatty acids.

The alkaloids and sterols were separated chromatographycally from the lipophilic complex, utilizing the chromatographic technique in thin layer.

RESULTS AND DISCUSSIONS

1. Chorology

Nigella arvensis L. is a Pontic-Mediterranean species, met spontaneously in Romania. Of the four subspecies described by STRID (197o), only N.arvensis L.ssp. arvensis was met in Romania. It is widespread on plain and hilly region (up to 3oo m S.m.) in either sides of the Carpathian's (fig.1), on cultivated grounds, ruderal places, dry meadows, mostly on sandy and clay-sandy soils, rendered obvious in the following vegetable formations:

Cl.SECALIETEA Br.-Bl. 1951 (segetal associations): ass. Tribulo-Tragetum Soó et Timár 1945; Aristolochio-Convolvuletum arvensis Ubrizsy 1965; Consolido (orientali) - Vicietum striatae (Slavič, 1944) Soó 1947 biforetum radiantis;

Cl.CHENOPODIETEA Br.-Bl. 1951 em Soó (ruderal associations): ass. Carduetum acanthoidis Morariu, 1939;

Cl.FESTUCO-BROMETEA Br.Bl. et Tx.1943 (xerophilous meadows): ass. Andropogonetum ischaemi Krist, 1937; Plantaginetum indicae Păun,1964; Trifolietum echinati Morariu 1969; Artemisiae (austriacae) - Poëtum bulbosae I.Pop 1971; Convolvuletum lineatus Morariu 1967.

Nigella damascena L. is cultivated as an ornamental plant (for flowers and fruits) since the Middle Ages. "Single flower" form was represented by the native population with flowers having a colour ran-

ge from blue to white) and an Utrecht (Holland) population with white flowers. The "double flower" form presents a recessive gene (RAMAN, GREYSON, 1978) for stamins transformed in petals.

Nigella sativa L. is cultivated as an aromatic and medicinal plant, especially in the south-eastern Romania (Constanța and Brăila districts). Growing in a wild, it appears like a weed in the crops, especially in southern Romania.

2. Anatomy

Stem anatomy. In cross sections performed in the median zone and in the internode II under the flower, the stem of the analyzed species presents a primary structure. The stem epidermis is unistrate, having a cuticule outside. The bark is little developed and is presented under the form of assimilatory chlorenchyma. This one is continuous in N.arvensis arvensis, while in the other species it is interrupted, in front of the stem edges, by the angular collenchyms. The stele is well developed, with leading libero-ligneous fascicle of open collateral type. The form, number and dimensions of the sclerenchyma cords are characteristics for each analyzed species (Table 1). In the central zone of the sclerenchyma cords at the stem of the two N.damascena forms cells are to be found with thin walls, whose function is unclear for us.

Table 1.

Number and size of sclerenchyma cords in Romania's Nigella L. species

Species	Sclerenchyma cords		
	Number	Width (μm) $\bar{x} \pm s_{\bar{x}}$	Thickness (μm) $\bar{x} \pm s_{\bar{x}}$
Stem median zone			
N. arvensis arvensis	27	155.87 ± 18.00	139.35 ± 14.00
N. damascena "single flower"	25	148.32 ± 11.75	118.92 ± 1.55
N. damascena "double flower"	25	139.56 ± 14.30	119.18 ± 11.80
N. sativa	21	187.55 ± 17.70	85.65 ± 10.10
Internode II zone			
N. arvensis arvensis	20	117.03 ± 15.48	79.38 ± 11.42
N. damascena "single flower"	14	106.95 ± 13.38	96.64 ± 14.43
N. damascena "double flower"	20	91.15 ± 8.69	98.54 ± 10.54
N. sativa	16	117.82 ± 13.57	83.72 ± 11.75

Structural characteristics of the leaf. The caulinare leaf is of equifacial type in N.arvensis arvensis, while the two form of N.damascena and N.sativa presents leaves of bifacial type. In all analyzed species, the palisade parenchyma is formed by two cell layers.

The epidermic cells are disposed unorderedly, having walls with different degrees of undulation (larger on inferior epidermis) and presenting dimensions characteristic for each analyzed species (Table 2).

The leaves of the analyzed species present, on both faces, stomata being higher on the inferior face (Table 2).

Table 2.

The size of the cauline leaf cells and the density of stomata in Romania's Nigella L. species

Species	Epiderma cells		Stomata density (number/mm^2)
	Length (µm)	Width (µm)	
	$\bar{x} \pm s_{\bar{x}}$	$\bar{x} \pm s_{\bar{x}}$	$\bar{x} \pm s_{\bar{x}}$
Upper epidermis			
N. arvensis arvensis	104.79 ± 5.12	44.83 ± 0.99	105.31 ± 4.67
N. damascena "single flower"	138.87 ± 3.89	64.69 ± 1.23	27.16 ± 1.08
N. damascena "double flower"	99.10 ± 2.50	50.27 ± 0.82	112.13 ± 4.71
N. sativa	126.64 ± 2.92	64.89 ± 1.57	50.94 ± 2.34
Lower epidermis			
N. arvensis arvensis	90.27 ± 0.55	39.64 ± 0.76	148.57 ± 5.89
N. damascena "single flower"	103.44 ± 3.41	59.87 ± 1.10	119.36 ± 4.88
N. damascena "double flower"	92.15 ± 2.34	45.98 ± 0.71	127.33 ± 5.23
N. sativa	114.36 ± 2.23	71.34 ± 1.73	89.30 ± 4.08

3. Chromosome characteristics. All the analyzed species are diploid (2n=12; n=6), the first five chromosome pairs being metacentric and the last pair (the smallest) acrocentric. Differences were found between the analyzed Nigella species, regarding the number and position of secondary constrictions, the value of arms ratio and the relative length of the chromosomes (Table 3). Nigella arvensis arvensis and N.damascena (both forms) present three chromosome pairs with satelli-

Table 3.

The characteristics of the chromosomes in the Romania's Nigella L. genus species.

Chromosome pair	N. arvensis arvensis			N. damascena "single flower"			N. damascena "double flower"			N. sativa		
	Chromosome length (µm)	Relative length (%)	Arms ratio	Chromosome length (µm)	Relative length (%)	Arms ratio	Chromosome length (µm)	Relative length (%)	Arms ratio	Chromosome length (µm)	Relative length (%)	Arms ratio
I.	9.70[x]	19.72	1.24	15.75	21.20	1.06	13.68	20.42	1.04	12.90	20.48	1.19
II.	9.08[x]	18.46	1.27	13.85[x]	18.64	1.27	12.40[x]	18.51	1.18	12.52[x]	19.88	1.46
III.	8.60[x]	17.48	1.30	13.10[x]	17.63	1.25	12.09[x]	18.05	1.16	11.95[x]	18.98	1.19
IV.	8.33	16.94	1.09	12.85[x]	17.29	1.06	11.56[x]	17.26	1.24	10.15	16.12	1.26
	7.88	16.02	1.06	12.20	16.42	1.03	10.90	16.27	1.06	9.55	15.17	1.12
[illegible]	5.60	11.38	9.18	6.55	8.82	8.36	6.36	9.49	8.94	5.90	9.37	7.53
Genome length (µm)	49.19	-	-	74.30	-	-	66.99	-	-	62.97	-	-

[x]Chromosomes with satellites

ten (the pairs I, II, III, respectively the pairs II, III, IV), whereas N.sativa presents only two chromosome pairs with satellites (the pairs II and III). N. damascena "double flower" usually has one of the chromosomes of the pair I comparatively longer as in its homologous (chromosomal rearrangements).

4. Genetic determinism of the flower colour. Flower colour in Nigella damascena "single flower" (spontaneous form) is determined by the presence of a gene with incomplete dominance, the gene with a pleiotropic action, affecting also the plant fertility. The gene presence in recessive homozygote state (aa) determines the white colour of the flowers (by the Utrecht population), in dominant homozygote state (AA) determines a deep-blue colour, and in heterozygote state (Aa) determines the colour of the petals in various hues of blue (the zone of the main nervures of the petal being blue, and towards the extremely of the petals turns step by steps into white by the Crimea population).
The segregation report interpreted on the basis of χ^2 test confirmed mendelian segregation (CCL KMU, 1974).

The gene presence in a recessive homozygote state (aa) in N.damascena "double flower" (white flowers) determines complete sterility (CCL KMU, 1974).

5. Genetic modification in the descent of the irradiated seeds. Albinotic individuals (X_1) developed from N.damascena seeds which received 100 K fission neutrons dose were crossed and produced in X_2 generation, normal, non-viable and phyllodic offspring.

The phyllodic aspect (N.damascena phyllodia) which occured in 1.5 % of the offsprings of the albinotic individuals, caused slightly smaller plants with smaller, less lobed leaves that detached before the plant matured. The cauline leaves were reduced in number, and the floral elements were transformed into leaflets on a limb of 4-5 mm long.

The forward-mutation and the back-mutation for the flower shape (N.damascena) from the typical species ("single flower") to the horticultural form ("double flower") and inversely. Seeds of N.damascena "Miss Jekyll" exposed to 400 K fission neutrons, exhibited back mutation to the flower type of N.damascena "single flower" at the rate of 5 %, whereas N.damascena "single flower" seeds exposed to the same dose produced flowers of the "Miss Jekyll" type ("double flower") at the rate of 1 out of 12.5 plants. In the second generation of the "Miss Jekyll" type, the flowers of the plants were sterile, with varying flower shape

6. Seed chemical composition. In the seeds of the analyzed Nigella species and populations the following chemical compounds were identified: base alkaloids and salt alkaloids, volatile oils, free and combined fat acids, organic acids, glicerides or sterides, sterols, phytosterols or phytosteroides, reducing monozes and mucilages.

Analyzis of the lipophilic complex from the seeds (Table 4), revealed that N.damascena and N.sativa though situated in different sections in the A.TERRACCIANO classification, present the same number of alkaloids (2), free fat acids (3) and combined fat acids (2), having a different chemical composition with N.arvensis arvensis (1, 2 respectively 2).

The Rf values and their spot colour in time revealed that the sterols with Rf=0.62 are identically in N.arvensis arvensis and in N. damascena populations, and the sterols with Rf=0.98, respectively 0,97 from N.arvensis arvensis and N.sativa are probably similar (the spot of violet colour), being different from sterols with Rf=0.97 from N.damascena populations (brown-violet spot).

Table 4.

Study of lipophilic complex obtained from the seeds of some Nigella genotypes

Species	N.arvensis arvensis		N.damascena -Craiova		N.damascena - Utrecht		N.damascena "Miss Jekyll"		N.sativa	
Chemical compounds	Spot number	Rf	Spot number	Rf	Spot number	Rf	Spot number	Rf	Spot number	Rf
Unsaponificable fraction										
Alkaloids[x]	1	0.77	2	0.79 0.83	2	0.79 0.83	2	0.79 0.83	2	0.[illegible] 0.[illegible]
Alkaloids[xx]	1	0.63	2	0.69 0.72	2	0.69 0.72	2	0.69 0.72	2	0.[illegible] 0.[illegible]
Sterols	3	0.56 0.62 0.98	2	0.62 0.97	2	0.62 0.97	2	0.62 0.97	1	0.[illegible] 0.[illegible] 0.[illegible] 0.[illegible]
Saponificable fraction										
Free fat acids	2	0.49 0.71	3	0.61 0.73 0.91	3	0.41 0.55 0.83	3	0.50 0.67 0.90	3	0.[illegible] 0.[illegible] 0.73
Combined fat acids	2	0.50 0.71	3	0.61 0.73 0.90	2	0.55 0.83	3	0.67 0.81 0.90	3	0.[illegible] 0.[illegible] 0.73

[x]Solvent system: acetone : toluene : methanol : ammonia 25%

[xx]Solvent system: chlorepherm : ethanol : water

Between the three Nigella species, as well as between the three N.damascena populations differences were found in number and Rf values, both in the free fatty acids and in the combined fatty acids (Table 4).

Comparing the plant Rf values (Table 4) with Rf values of some fatty acids utilized as reference substances (Table 5), some free fatty acids were identified in the analyzed genotypes (Table 5).

N.damascena population from Utrecht with white flower present two combined fatty acids, while the spontaneous form with blue flowers presents three combined fatty acids.

Table 5

The features of some fatty acids utilized as reference substances

Fat acid	Rf	Present in seeds in:
Stearic acid	o.49	N.arvensis arvensis N.damascena "Miss Jekyll" (?)
Linolic acid	o.55	N.damascena - Utrecht
Palmitic acid	o.62	N.damascena - Craiova (?) N.sativa (?)
Undecilenic acid	o.82	N.damascena - Utrecht (?)
Caprilic acid	o.9o	N.damascena "Miss Jekyll" N.damascena - Craiova (?)

Stationary phase: chromatographic paper Whatmann 1
Solvent system: glacial acetic acid
Developing time: 2 h at 24°C
Revealing with saturated solution of copper and solution 7.5 % of potassium ferocianure.

CONCLUSIONS

1. In the Romania's flora, the Nigella L.genus is represented by three species: N.arvensis L. ssp.arvensis present in spontaneous flora, N.damascena L. (with several cultivated varieties) and N.sativa L. are especially cultivars (ornamental, aromatic and medicinal plants).

2. All the analyzed species present in the stem a primary structure. The number and the dimensions of the sclerenchyma cords are characteristics for each analyzed species.

3. The cauline leaf is of equifacial type in N.arvensis arvensis while N.damascena and N.sativa presents leaves of bifacial type. All the three species presents stomata of anomocytic type. The form, disposal

and the dimensions of the epidermic cells on both faces of the leaves are characteristics for each analyzed species.

4. All the analyzed species are diploid (2n=12), with differences in the chromosome length, arms ratio and chromosome pairs which bear satellites.

5. The flower colour in N.damascena "single flower" is determined by a pleiotropic gene with incomplete dominance.

6. The study of the irradiated descend revealed phenotypic mutants: "phyllody mutant" in N.arvensis arvensis; "forwardmutation" and "back-mutation" for the flower shape in N.damascena.

7. The analysis of the seed chemical composition revealed that all the studied Nigella species and populations contained the same group of substances. Differences have been found regarding the contents in alkaloids, sterols, free and combined fatty acids.

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REZUMAT

CORNEANU G.C., POPESCU M., SITORIS A., ȘTEFANU V.D., POPESCU G., 1986, Cercetări privind biologia speciilor genului Nigella L. (Fam. Ranunculaceae) din România. Not.bot.hort.agrobot., Cluj, XVI., 151-163.

Lucrarea prezintă un istoric și un studiu biologic complex asupra speciilor genului Nigella L. care vegetează în România: N. arvensis L. ssp. arvensis, N. damascena L. și N. sativa L. N. arvensis arvensis este întîlnită în flora spontană în asociații segetale, ruderale și xerofile, iar N. damascena și N. sativa sînt cultivate (sporadic) ca plante medicinale, aromatice sau decorative. Studiul anatomic a evidențiat caracteristicile structurale ale tulpinii și frunzei (tulpina cu structură primară; frunză de tip equifacial la N. arvensis arvensis și de tip bifacial la N. damascena și N. sativa; stomate de tip anomocitic ș.a.), precizîndu-se caracteristicile structurale ale fiecărui genotip. Analiza numărului somatic de cromosomi a evidențiat că toate speciile analizate sînt diploide (2n=12, x=6), între ele constatîndu-se diferențe în mărimea relativă a cromosomilor, raportul brațelor, numărul perechilor de cromosomi care poartă sateliți. Culoarea florii la N. damascena "single

flower" este determinată de o genă pleiotropă cu dominanţă incompletă. Studiul descendenţei plantelor iradiate a evidenţiat unele mutante somatice: mutanta "phyllody" la N. arvensis arvensis, "mutaţia-înainte" şi "mutaţia-înapoi" pentru forma florii la N. damascena (de la forma spontană "single-flower" la forma cultivată "double-flower" şi invers). Studiul compoziţiei chimice a seminţelor a evidenţiat prezenţa (la speciile analizate) aceloraşi grupe mari de substanţe: alcaloizi [illegible] alcaloizi săruri, uleiuri volatile, acizi graşi liberi şi combinaţi, acizi organici, gliceride sau steride, steroli, fitosteroli şi fitostereizi, meneze- oze reducătoare şi mucilagii. Între genotipurile analizate s-au constatat diferenţe în conţinutul în alcaloizi, steroli, acizi graşi liberi şi combinaţi. S-a constatat implicaţia acizilor graşi combinaţi în determinismul culorii florii, sub control genetic, la N. damascena (ipoteză).

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