

Gamma-rays induced reciprocal translocation in *Nigella damascena* L. (Love-in-a-mist)

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Abstract — Gamma irradiations (5, 10, 15 and 20 kR) to *Nigella damascena* L. (love-in-a-mist; family: Ranunculaceae) seeds (moisture content: 8.7%) induced three viable translocation heterozygotes (P-3 and P-15 from 5 kR and P-25 from 10 kR) which on selfing yielded progeny heterozygotes in subsequent generations (R_2 , R_3 and R_4 ; however, P-15 could only be assessed upto R_2 generation) exhibiting the formation of either a ring or a chain of four chromosomes in 14.8-54.6% meicytes. Mean quadrivalent frequency per cell was assessed to be 0.40-0.66 in P-3, 0.43-0.64 in P-15 and 0.15-0.47 in P-25. Configuration and orientation of the multiples in the heterozygotes assessed over the generations indicated a possible genetic control over the mechanism. Normal 6/6 separation of chromosomes at AI (55.86-100.00%) of the heterozygotes did not correlate significantly ($r = 0.29$, 27 DF) with pollen fertility (6.98-46.38%). Pollen fertility showed negative and insignificant correlation ($r = -0.20$, 27 DF) with seed set per plant (0.0-495.0). Total failure of seed setting in heterozygotes has been attributed to defective female gametogenesis. F_1 s raised from intercrossing (R_1) of P-3, P-15, and P-25 were meiotically assessed and the results indicated that the same two non-homologous chromosomes were involved in translocation and the two longest chromosome pairs A_1A_1 (nucleolar pair) and A_2A_2 were suggested to be associated.

Key words: chromosome identification; gamma-rays; *Nigella damascena*; progeny analysis; translocation heterozygotes.

INTRODUCTION

Nigella damascena L. (love-in-a-mist), a member of the family Ranunculaceae, is an ornamental plant and is a good material for cytological studies as it has relatively low number of chromosomes ($2n = 12$) of suitable sizes and good stainability and possesses graded karyotype with a marker telocentric pair (DATTA and SAHA 2003). Such cytological novelty can be explored for understanding cytogenetical behaviour of plant types of interest (DATTA *et al.* 2003; GHOSH and DATTA 2004). Present authors have induced three viable translocation heterozygotes in *N. damascena* following gamma irradiations (GHOSH and DATTA 2003) as a part of research initiated in the species for improvement by the manipulation of genetic and cytogenetic consequences of induced mutagenesis and this paper describes comprehensive meiotic chromosome behaviour of the translocation heterozygotes assessed over the generations based on

configuration and orientation of the interchanged multiples and their subsequent effect on fertility. Further, meiotic plates have been used to identify the chromosomes involved in translocation following karyotype analysis. Reciprocal translocation is an important tool for chromosome manipulation and viable translocation heterozygotes have immense value in breeding behaviour of the crop besides its utmost significance in understanding gene and chromosome relationship of the species.

MATERIAL AND METHODS

Plant material - Dry seed of *Nigella damascena* L. (mother seed stock obtained from Royal Botanic Garden, Kew, London — accession no. 0016287) having 8.7% moisture content were exposed to gamma-rays (5, 10, 15 and 20 kR from ^{60}Co source at the rate of 1kR for 2 mins. 24 secs.) and three translocation heterozygotes (Plant no. 3 and 15 from 5 kR and P-25 from 10 kR) were screened from R_1 population following meiotic analysis (PMCs and pollens were stained in 1% propionocarmine solution; fully stained pollens were considered fertile).

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Chromosome studies - Selfed progenies of each heterozygote in subsequent generations (R_2 , R_3 and R_4) were meiotically assessed (25 seeds from each heterozygote were sown in separate lines in each generation) and the translocation heterozygotes were identified and their cytological behaviour (configuration and orientation of the multiples and equal 6/6 anaphase I frequency), pollen fertility (PF) and seed set (S) per plant have been studied and presented in Chart 1. P-15, however, could only be studied upto R_2 generation.

Evaluation of translocation heterozygotes were made on the basis of configuration (> ring — **R**, > chain — **C**, ring and chain in equal frequency — **RC**) and orientation (> adjacent — *ad*, > alternate — *al*, and random — *r*,) of the interchanged complexes occurring in the meiocytes at diplotene and metaphase I. X^2 -test analysis has been performed to ascertain the configuration and orientation of the multiples in a heterozygote. Multiple were assigned as alternate or adjacent from the proposed configuration of BURNHAM (1956) and SYBENGA (1975).

For identification of the chromosomes involved in translocations, the viable heterozygotes were intercrossed (P-3 x P-15, P-3 x P-25, P-15 x P-25) at R_1 and F_1 s raised (excluding reciprocals) were examined meiotically (2-3 plants were examined from each line).

Mitotic chromosome preparation was made following DATTA and SAHA (2002) and properly condensed metaphase plates were assessed through Micro Image™ Lite Software (Version 4.0 for windows). Idiogram was constructed using Adobe Photoshop 7.0 Version to ascertain the chromosome types which could easily be identified and marked in most of the meiotic plates.

RESULTS AND DISCUSSION

Mitotic chromosome type in N. damascena - Three morphologically distinct chromosome types (A, B and C) have been recorded (Figs. 1 and 2) in control plants ($2n = 12$) on the basis of chromosome length (A-long chromosomes = 13.0 to <15.0 μm ; B-medium = 7.0 to <13.0 μm and C — short = less than 7.0 μm), nature of primary constrictions and presence or absence of secondary constrictions. The somatic complement possessed 1 pair A_1A_1 (absolute length 13.96 μm , F% 41.46; with satellites), 1 pair A_2A_2 (absolute length 13.62 μm ; F% 46.46), 1 pair A_3A_3 (absolute length 13.07 μm ; F% 40.04; with satellites), 2 pairs of BB (B_1B_1 — absolute length 11.78 μm ; F% 44.93 and B_2B_2 — absolute

length 11.18 μm ; F% 43.54) and 1 pair CC (absolute length 6.53 μm ; F% 14.59) chromosomes. These chromosomes could easily be identified and marked in meiotic plates of translocated plants (Figs. 4-6). Previously, DATTA and SAHA (2003) reported four chromosome types (A-D) in the species.

Meiotic analysis - The average chromosome association per cell assessed over the generations in control plants (Fig. 3) have been 5.88 II + 0.25 I ($2n = 12$, 120 PMCs scored). The marked plants (P-3, P-15 and P-25) obtained through gamma irradiations and their heterozygote progenies (1 mostly and rarely 2 to 5 from 20.0 to 32.0 per cent germinated plants in each line) exhibited the formation of ring or a chain of 4 chromosomes (Figs. 4-6) in 14.8% to 54.6% meiocytes ($2n=12$) indicating well organised reciprocal exchanges involving 2 non-homologous chromosomes. Mean quadrivalent frequency per cell in heterozygote lines (P-3: 0.40-0.66, P-15: 0.43-0.64 and P-25: 0.15-0.47) was non random when assessed over the generations ($X^2 = 21.86$, 2DF, P value > 0.001) but was random among the plants of P-3 assessed at R_4 ($X^2 = 11.98$, 8 DF, p value 0.10 to 0.20) and among floral buds of a heterozygote of P-3 line (P value 0.10-0.40) as evidenced from X^2 — test of heterogeneity. Predominant association noted among the heterozygote lines has been 1 IV + 4 II (P-3: 33.7-62.3%; P-15: 29.1-63.9%; P-25: 13.3-40.0%).

Configuration of the multiple in induced heterozygotes (R_1) were random but their progenies in subsequent generations demonstrated preponderance of either ring (R) or chain (C) or random (RC) configurations in meiocytes. Progeny heterozygotes having significantly higher frequency of ring or chain quadrivalents in PMCs gave rise to progenies with greater ring or chain multiple respectively. Rings (Figs. 4 and 6) were found when the interchanged parts of the chromosomes were long and more or less equal in length and the break points was closer to centromere; while, a chain (Fig. 5) of 4 chromosomes was formed when the exchanged chromosomal piece was relatively short (BURNHAM 1956; BURNHAM and HAGBERG 1956). KAUL (1977) was of opinion that the breakage and exchange of heterologous chromosomes are genetically conditioned and controlled, thereby eliminating the 'chance factor' operating for the predominance of either ring or chain or both. In the present investigation, orientation of multiples were either random (*r*) or more of adjacent type (*ad*). Heterozygotes with random orien-

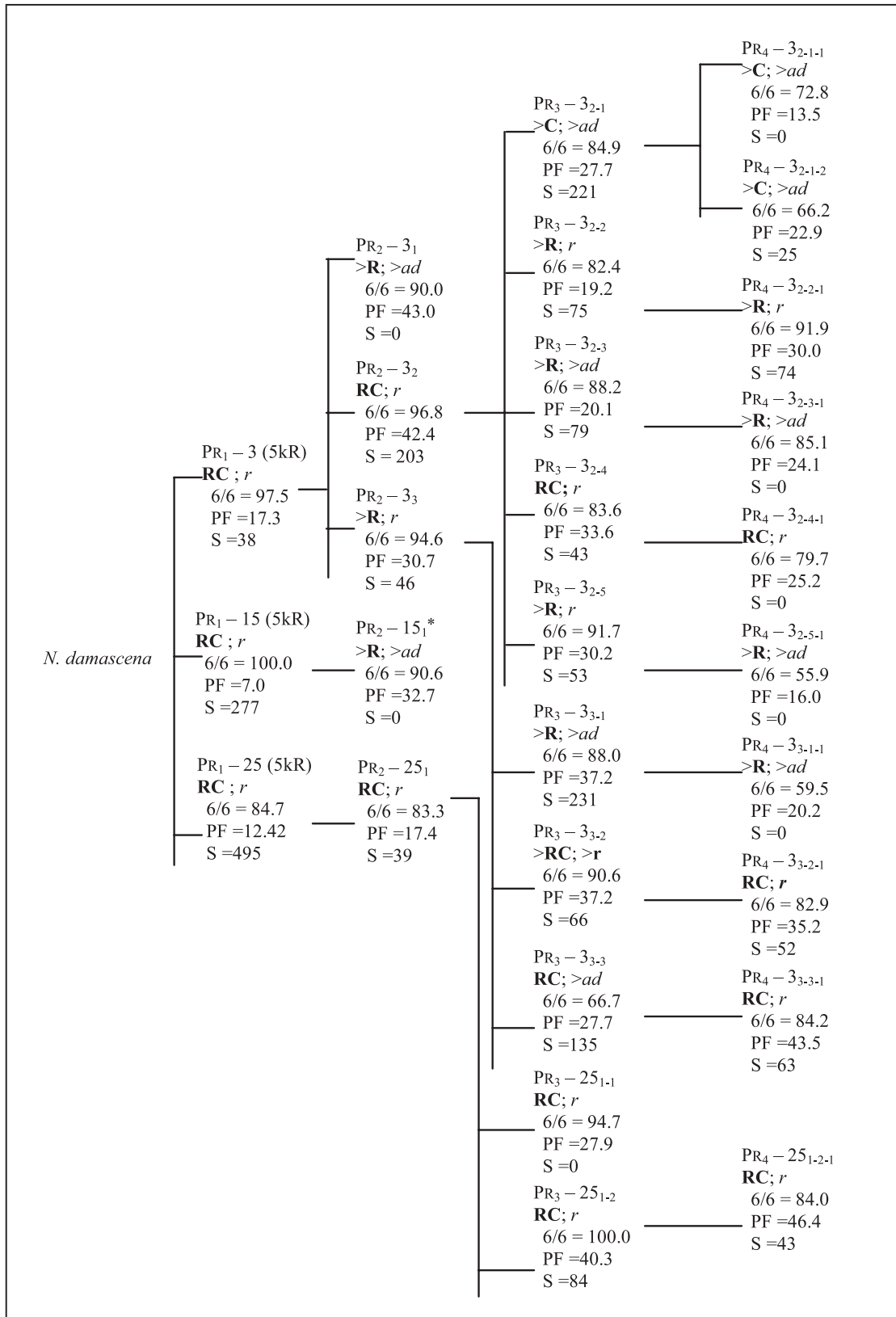
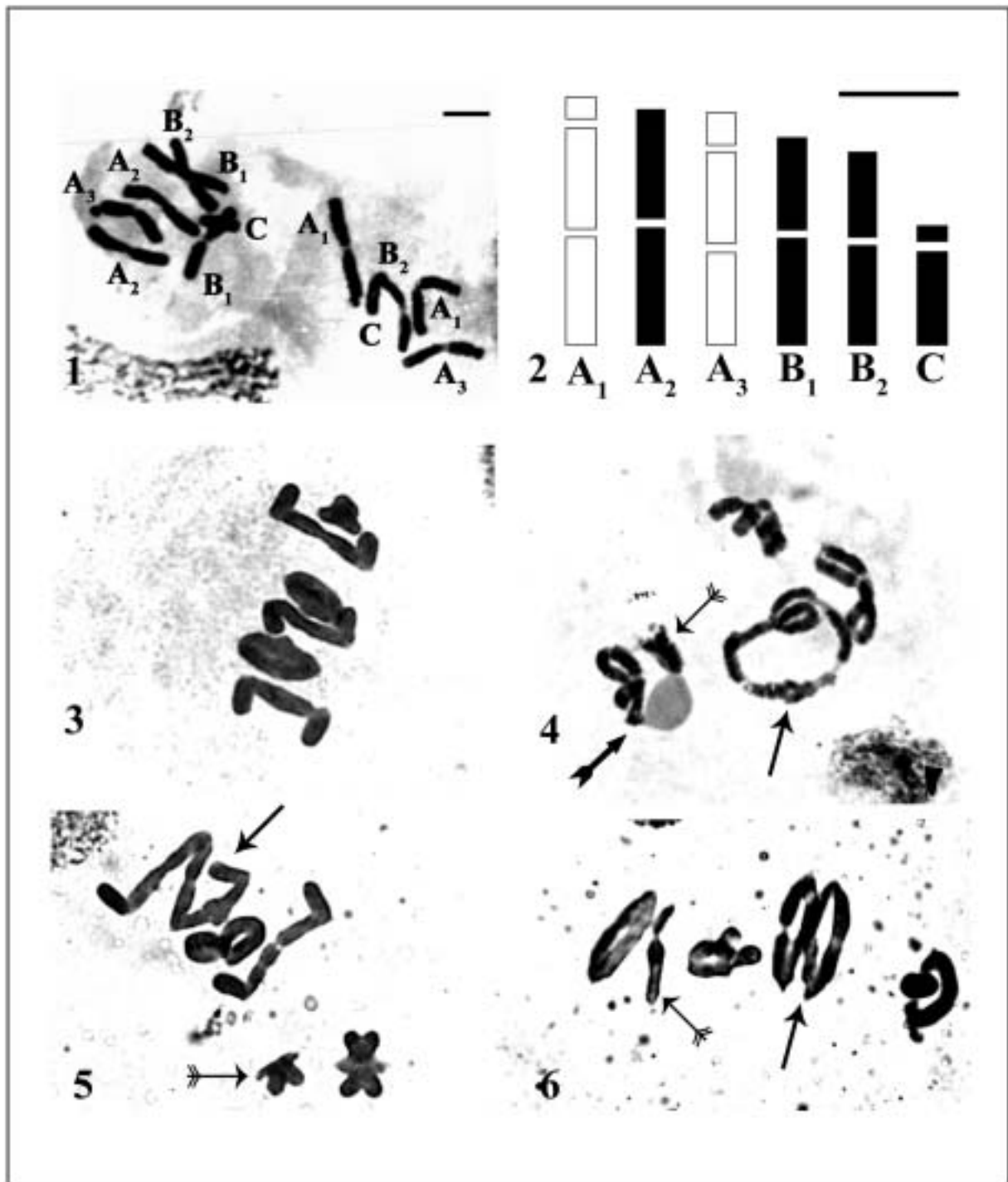


Chart 1 — Translocation heterozygotes at different generations showing configuration (bold) and orientation (italics) of multiple with frequency of 6/6 AI cells, pollen fertility (%) and seed set per plant; * fruit-setting but seedless.



Figs. 1-6 — Mitotic (1-2) and meiotic (3-6) chromosomes ($2n = 12$) in *Nigella damascena*. 1) Metaphase showing chromosome types. 2) Idiogram. Bar in mitotic plates = $6.527 \mu\text{m}$. 3) 6 II at metaphase I. 4) 1 IV + 4 II at diplotene. 5-6) 1 IV + 4 II at MI with alternate orientation of chain (5) and ring (6) multiples. Interchanged configurations (\rightarrow), $A_3 A_3$ (\Rightarrow) and CC (\Leftrightarrow) pairs have been marked in meiotic plates.

tation gave rise to progenies with random and adjacent types; while, adjacent type produced only adjacent orientation (exception: PR_4-3_{3-3-1} and PR_3-3_{3-3}). Rings tended to orient as adjacent whereas chains had a more frequent alternate orientation. Therefore, analysis of heterozygotes in relation to configuration and orientation of the multiples assessed from self segregating lines over the generations indicated a possible genetic control over the mechanism. Such configuration and orientation of the multiples have also been documented in viable translocation lines of *Nigella sativa* (SAHA and DATTA 2002). This explains the deviations from expectation that reciprocal translocation in a particular species shows either random or directed (significantly more than 50% meiocytes showing alternate configurations) orientation of the interchanged complexes at metaphase I but not both (BURNHAM 1956).

Genetic control of alternate segregation has been evidenced by THOMPSON (1956 a; b) and LAWRENCE (1958) in rye. On the contrary, orientation of interchanged complex have been considered to be mechanical in nature with genetic control being the secondary determinant factor (GARBAR and DHILLON 1962; ZAMAN and RAI 1972). Greater flexibility of chain quadrivalent resulting mostly in alternate orientation (OSTERGREN 1951; LEWIS and JOHN 1963) supported the mechanical hypothesis.

High frequency of normal 6/6 separation of chromosomes at anaphase I of the heterozygotes (55.86% to 100.00%) did not correlate significantly ($r = 0.29$, 27 DF) with pollen fertility (6.98% to 46.38%), thereby, indicating that all cytologically balanced AI cells were surely not genetically balanced. Although predominant occurrence of adjacent orientation in quadrivalents in heterozygotes is expected to induce high frequency of non-viable male gametes following duplication and deficiency of genes, only some of them were seedless irrespective (seed set in heterozygotes — 0 to 495/plant) of their pollen fertility (pollen fertility and seed set in heterozygotes showed negative and insignificant correlation between them — $r = -0.20$, 27 DF). Further, two heterozygotes ($PR_3 - 25_{1-1}$ and $PR_4 - 3_{2-4-1}$) having random orientation of the multiple were also seedless. These plants failed to produce seeds on open or controlled pollinations. Thus, failure in seed setting in heterozygotes may not be the outcome of orientation of multiples but may be associated with defective female gametogenesis.

Meiotic analysis of intercrossed progenis at F_1 demonstrated the formation of one ring quadriva-

lent in association of bivalents and univalents at MI ($2n = 12$) in 54.84% (P-3 x P-15 – 62 PMCs scored), 45.63% (P-3 x P-25 – 103 PMCs observed) and 55.17% (P-15 x P-25 – 87 PMCs studied) cells; while, the rest had bivalents and univalents only. Results of the intercrossing suggested that the same two pairs of non-homologous chromosomes were involved in translocation and from the chromosome types it may be inferred that the two longest pairs A_1A_1 (nucleolar pair) and A_2A_2 were associated.

Although detectable phenotypic marker could not be assigned to the heterozygotes, viable translocation lines of P-3 and P-25 may further be exploited for valuable cytogenetical information in the species.

REFERENCES

- BURNHAM C. R., 1956 — *Chromosomal interchanges in plants*. Bot. Rev., 22: 419-552.
- BURNHAM C. R. and HAGBERG A., 1956 — *Cytogenetic notes on chromosomal interchanges in barley*. Hereditas, 42: 467-482.
- DATTA A. K., GHOSH A. and SENGUPTA S., 2003 — *Highly unstable male meiosis in two aberrant plants isolated from the mutant population of Nigella damascena L. (love-in-a-mist)*. Cytologia, 68: 383-388.
- DATTA A. K. and SAHA A., 2003 — *Cytomorphological studies and seed protein characterization of Nigella sativa L. and Nigella damascena L.* Cytologia, 68: 51-60.
- GARBER E. D. and DHILLON T. S., 1962 — *The genus Collinisia XVIII. A cytogenetic study of radiation-induced reciprocal translocations in C. heterophylla*. Genetics, 47: 561-567.
- GHOSH A. and DATTA A. K., 2003 — *Cytogenetical studies on the induced viable translocation lines of Nigella damascena L. (Love-in-a-mist)*. Cell and Chrom. Res., 23: 5-10.
- GHOSH A. and DATTA A. K., 2004 — *A report on gamma-rays induced paracentric inversion in Nigella damascena L. (love-in-a-mist) involving marker telocentrics*. Cell and chrom. Res., 24: 7-11.
- KAUL M. L. H., 1977 — *Cytogenetics of white Allium I. Translocation heterozygosity*. Cytologia, 42: 681-689.
- LAWRENCE C. W., 1958 — *Genotypic control of chromosome behaviour in Rye VI. Selection for disjunction frequency*. Heredity, 12: 127-131.
- LEWIS K. R. and JOHN B., 1963 — *Chromosome Marker*, J. A., Churchill Ltd., London.
- OSTERGREN G., 1951 — *The mechanism of co-orientation in bivalents and multivalents. The theory of orientation by pulling*. Hereditas, 37: 473-494.

- SAHA A. and DATTA A. K., 2002 — *Gamma-rays induced reciprocal translocation in Black Cumin (Nigella sativa L.)*. Cytologia, 67: 389-396.
- SYBENGA J., 1975 — *Meiotic configurations – A source of information for estimating the genetic parameters*. Springer-Verlag, Berlin, p. 210-214.
- THOMPSON J. B., 1956a — *Genotypic control of chromosome behaviour in rye II. Disjunction at meiosis in interchange heterozygotes*. Heredity, 10: 99-108.
- THOMPSON J. B., 1956b — *Genotypic control of chromosome behaviour in rye VI. Selection and disjunction frequency*. Heredity, 10: 127-131.
- ZAMAN M. A. and RAI K. S., 1972 — *Cytogenetics of thirteen radiation induced reciprocal translocations in Collinsia heterophylla*. Cytologia, 37: 629-638.

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