RESEARCH ARTICLE

KARYOTYPIC STUDIES ON *TOXOPTERA AURANTII* AND *MYZUS PERSICAE* INFESTING ROSE PLANTS FROM HIMACHAL PRADESH, INDIA

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SUMMARY Karyotypic studies on two species of aphids, *Toxoptera aurantii* (Boyer de Fonscolombe) and *Myzus persicae* (Sulzer) infesting rose plants from Himachal Pradesh were carried out. Both these species infesting rose plants from Himachal Pradesh are observed for the first time. The diploid chromosome numbers in these species were found to be 2n = 8 and 2n = 12 respectively. The chromosomes were holocentric. Total complement length as well as relative lengths of chromosomes were calculated. Idiograms of both the species showed a gradual decrease in the size of chromosome pairs.

Keywords: Karyotypes, Toxoptera, Myzus, holocentric chromosomes, total complement length, idiograms.

INTRODUCTION

At present, chromosome numbers of 1039 species of aphids belonging to 14 families are known, which comprise about 24% of the total number of aphid species (Gavrilov-Zimin et al. 2015). About 250 aphid species are considered serious pests of crops and cause great losses of yield (Remaudiére & Remaudiére 1997).

Aphids have complex life cycles involving thelytokous parthenogenesis, polyphenism, viviparity, telescoping of generations and host alternation. Interest in aphid cytogenetics is mostly due to the holocentric nature of chromosomes (Wrensch et al. 1994). Chromosomal polymorphism occurs in aphids as a result of fusion or fission of chromosomes which leads to evolution of new biotypes (Blackman 1980, Gautam et al. 1993).

Rose is one of the most beautiful ornamental flowers. It is attacked by numerous pests; amongst

them, aphids are considered as a major pest. It is infested by 55 aphid species (Blackman & Eastop 2000), of which 39 are previously recognized from India (Raychaudhuri 1983, Chakrabarti & Sarkar 2001). Since rose aphids are of particular importance because of the damage caused and not much work has been done on chromosomes of rose aphids from Himachal Pradesh. Hence, the present investigation was undertaken to study the chromosomes of two species of aphids from this region.

Genus *Toxoptera* belongs to tribe Aphidini of family Aphididae and includes 4 or 5 species resembling *Aphis*. Genus *Myzus* belongs to tribe Macrosiphini of family Aphididae and includes about 55 species. The primary hosts of this heteroecious species are *Prunus* spp., but the secondary hosts belonged to many different families. The present paper deals with the karyotypes of *T. aurantii* (Boyer de Fonscolombe) and *M. persicae* (Sulzer).

MATERIALS AND METHODS

For chromosomal studies, aphids were collected from leaf and flower buds, underside of young leaves and along green stems of rose plants from different localities of Himachal Pradesh (30° to 33° N latitude and 75° to 79° E longitude). Aphids of *T. aurantii* were collected from Alampur, Kangra (altitude 1254 m above sea level) and *M. persicae* were collected from Pragpur, Kangra (altitude 650 m above sea level). The infested plant parts were put in small polythene bags and brought to laboratory for cytological studies.

The embryos were taken out by puncturing the posterior end of the abdomen of an adult parthenogenetic female aphid. Then, embryos were pretreated in 0.7% sodium citrate solution for 30 min. The pretreated embryos were fixed in 1:3 acetic-ethanol solution for about 15–20 min at room temperature. After fixation, embryos were squashed on a glass slide in a drop of 45% acetic acid for 3–5 min and stained with 2% Giemsa for about 25–30 min followed by mounting in DPX. The slides were then thoroughly scanned under research binocular microscope and photomicrographs were taken. Well spread metaphase plates were selected for chromosome measurements. Actual lengths of chromosomes were measured using ocular micrometer. From actual lengths, the total complement length (TCL) was calculated for each species. From actual length data, the relative lengths of chromosomes were calculated. Chromosome pairs were then arranged in decreasing order of their lengths to prepare the karyotype of each species.

OBSERVATIONS

In the present study, chromosomes of two species of aphids namely, *T. aurantii* and *M. persicae* infesting cultivated *Rosa* spp. have been studied.

T. aurantii

This species has diploid chromosome number of 8 (Figs 1, 2). The length of chromosomes ranged from 1.41 μ m to 2.81 μ m. TCL was 16.94 μ m. The relative length of chromosomes ranged from 8.40 to 16.69. The somatic complement consists of 4 pairs of holocentric chromosomes showing a gradual decrease in their lengths (Fig. 3).



Figs 1–6: Karyotypes of aphids. 1–3. T. aurantii 1. Somatic chromosomes 2. Karyotype. 3. Idiogram. 4–6. M. persicae 4. Somatic chromosomes. 5. Karyotype. 6. Idiogram.

M. persicae

This species has diploid chromosome number of 12 (Figs 4, 5). The length of chromosomes ranged from 1.47 μ m to 3.83 μ m. TCL was 31.77 μ m. The relative length of chromosomes ranged from 4.59 to 12.10. The somatic complement includes 6 pairs of holocentric chromosomes which show a gradual decrease in their lengths (Fig. 6).

DISCUSSION

T. aurantii commonly known as black citrus aphid is polyphagous in nature. The diploid chromosome number of 2n = 8 reported here has been recorded by various workers from different host plants (Blackman 1980, Gautam & Dhatwalia 2003, Kar et al. 1990, Kar & Khuda-Bukhsh 1991, Pagliai 1961, Samkaria et al. 2010). The infestation of *T. aurantii* from rose plant was observed for the first time from Himachal Pradesh.

M. persicae commonly known as green peach aphid is extremely polyphagous in nature. The diploid chromosome number of 2n = 12 reported in the present study for *M. persicae* is in conformity with the earlier reports for this species by other workers (Chen & Zhang 1985, Dutta & Gautam 1993, Jangral et al. 2014, Sun & Robinson 1966, Wilson et al. 2002,). The infestation of *M. persicae* from rose plant was observed for the first time from Himachal Pradesh.

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REFERENCES

- BLACKMAN R L 1980 Chromosome numbers in Aphididae and their taxonomic significance Syst Entomol 5 7–25
- BLACKMAN R L & EASTOP V F 2000 Aphids on world's crops: an identification and information guide 2nd ed Chichester London
- J. CYTOL. GENET. VOL. 22 (NS) 1 & 2 (2021)

- CHAKRABARTI S & SARKAR A 2001 A supplement to the food-plant catalogue of Indian Aphididae (Homoptera) *JAphidology* **15**9–62
- CHEN X S & ZHANG G X 1985 The karyotypes of 51 species of aphids (Homoptera: Aphidoidea) in Beijing area *Acta Zoologica Sinica* **31** 12–19
- DUTTA J & GAUTAM D C 1993 Chromosomes of aphid fauna from North-western Himalayas India *Cytologia* **58**367–375
- GAUTAM D C & DHATWALIA N 2003 Karyotypes of twentyone species of aphids from North-western Himalayas *J Cytol Genet* **4** (NS) 1–9
- GAUTAM D C, CREMA R & BONVICINI PAGLIAI A M 1993 Cytogenetic mechanisms in aphids *Boll Zool* **60** 233–244
- GAVRILOV-ZIMIN I A, STEKOLSHCHIKOE A V & GAUTAM D C 2015 General trends of chromosomal evolution in Aphidococca (Insecta Homoptera Aphidinea+Coccinea) Comp Cytogenet **9** 335–422
- JANGRAL S, TRIPATHI N K & POONAM 2014 Chromosomal studies on two species of aphids from Jammu (J&K) India Int J Recent Sci Res 5 1338–1341
- KAR I & KHUDA-BUKHSH A 1991 Intra-specific karyotypic variations in two species of polyphagous aphids (Homoptera Aphididae) Entomon 16 139–146
- KAR I, BASU G & KHUDA-BUKHSH A R 1990 A checklist of chromosomes of aphids (Homoptera: Aphididae) worked out in India along with the names and families of their host plants *Environ Ecol* **8**414–428
- PAGLIAI A M 1961 L' Endomeiosi in *Toxoptera aurantii* (Homoptera: Aphididae) *Renticonti Classe di Scienze fisiche, matematiche e naturali* ser VIII **31** 455–457
- RAYCHAUDHURI D N 1983 Food-plant Catalogue of Indian Aphididae Aphidological Society of India Graphic Printall Kolkata
- REMAUDIÈRE G & REMAUDIÈRE M 1997 Catalogue of the World's Aphididae Homoptera: Aphidoidea INRA Paris

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- SAMKARIA R, BALA J & GAUTAM D C 2010 Karyotype studies on some commonly occurring aphid species *Nucleus* **53** 55–59
- SUN R Y & ROBINSON A G 1966 Chromosome studies on 50 species of aphids *Can J Zool* **44** 649–653
- WILSON A C C, SUNNUCK S P, BLACKMAN R L & HALES D F 2002 Microsatellite variation in cyclically

parthenogenetic populations of *Myzus persicae* in South-eastern Australia *Heredity* **88** 258–266

WRENSCH D L, KETHLEY J B & NORTON R A 1994 Cytogenetics of holokinetic chromosomes and inverted meiosis: keys to the evolutionary success of mites with generalization on eukaryotes *In* Houck M A (ed) *Mites: Ecological and Evolutionary Analysis of Life-History Patterns* Chapman and Hall New York pp 282–343

RESEARCH ARTICLE

STABILITY OF ONION HYBRIDS FOR YIELD AND MATURITY

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SUMMARY Investigation on identification of stable onion hybrids was worked out following Eberhart and Russell's model on 24 hybrids and 3 checks. Only hybrid, RH5 was stable for yield and maturity whereas RH2, RH6, RH10 and RH17 showed stability for yield but not for maturity as against stable commercial check, Virat. Hybrid, RH9 was found suitable for favorable environments while, RH12 and RH13 were good for poor environments (Rainfed) for yield. Neither white nor yellow hybrids withstood the test of stability. Molecular analysis confirmed the hybridity of RH5.

Keywords: Onion, Allium cepa, stability, hybrids, yield, maturity, molecular analysis.

INTRODUCTION

Onion (Allium cepa L.) is one of the important export earning vegetable crops of India having a lot of demand by playing major role in earning foreign exchange and also good demand internally due to high consumption rate. Its cultivation is extensive during kharif, late kharif and rabi in parts of Maharashtra, Gujarat, Karnataka and Andhra Pradesh (Khar et al. 2007). Onion area under world is around 42 lakh hectares with the production of 7, 98, 00,000 t and productivity of 19.70 t/ha as against India having 12 lakh hectares with the production of 2, 18, 52,000 t and productivity of 14.21 t/ha. Karnataka is having an area of 1.4 lakh hectares with the production of 16, 80,000 t and productivity of 12 t/ha (Anonymous 2019), which is far below the productivity of advanced countries (45-50 t/ha); the reason being the use of open pollinated varieties. Many private and public sector organizations are involved in development of onion varieties in India but there

is limited work on development of commercially viable hybrids in onion. The advantage of hybrid breeding in onion is uniformity in bulb shape, earliness and high yield. In this context there is a need of locally available cytoplasmic male sterile (CMS) lines and maintainer lines with suitable restorers to produce the hybrids. Such hybrids should be commercially viable only if they are stable in yield and quality at varied production areas (Madalageri et al. 1998). Keeping this in view, the project was started in 2016 at I and B Seeds Private Limited, Bengaluru on red, white and yellow onions. The previous work on onion research at I and B Seeds, Bengaluru that developed male sterile lines and their maintainers on red, yellow and white onions which enabled to produce hybrid seeds with the use of diverse restorer lines as reported by Pujara et al. (2017). The present paper deals with statistical analysis concerning the yield and maturity of 24 hybrids and 3 checks maintained in I & B Farm, Bengaluru and molecular analysis of hybrid, RH5.

MATERIALS AND METHODS

Fortyfive d old seedlings of 24 onion hybrids with 3 checks were collected from nursery of I & B Farm, Bengaluru, transplanted on raised bed in Bengaluru and Ranebennuru while on flat beds in Devihosur (Haveri district). The experi-mental sites were geographically and agroclima-tically diverse wherein Bengaluru is situated at 920 m from MSL with 12.97 ° N altitude, 77.56° E longitude with 880 mm rainfall on red soils as against Ranebennuru and Devihosur of Haveri district with 630 m MSL, 14.47 °N latitude, 75.23 ° E longitude having annual rainfall of 700 mm on black soils. The trails were conducted in rainy, winter and summer seasons during 2020-2021 with 2 replications in randomized block design (RBD). The data were collected on various parameters. The ANOVA for each character was analyzed using standard statistical method while, stability was calculated for only yield and maturity following Eberhart & Russell's (1966) model. The data on yield was collected from 10 middle bulbs and averaged to calculate yield/bulb while, maturity was calculated at 50% of the population had neckfall.

Test of hybridity was done through molecular analysis using appropriate molecular markers. Leaf samples of parents and a hybrid (RH5) were collected from I & B Farm at 40 d after sowing. Plant genomic DNA was extracted using silica method (Jion-Feng et al. 2010). Hybrid and parents were screened using simple sequence repeats (SSR) marker ACM82 for differentiating male, female and hybrid DNA banding pattern. Polymerase chain reaction (PCR) was performed in 10 μ l reaction mixture containing 25 ng template DNA, 1 μ l 10X Taq polymerase buffer, 0.05 μ l of forward and reverse primer (0.5 μ M), 0.1 μ l of dNTPs (0.25 mM) each and 0.15 μ l (0.5 U) of Taq polymerase enzyme. PCR conditions were set according to the published literature. The PCR amplified fragments of varying sizes were separated using agarose gel electrophoresis and visualized with the help of Gel Doc after ethidium bromide staining. For marker ACM82 4% agarose gel was used to get clear separation.

OBSERVATIONS

24 hybrids and 3 checks of onion have been subjected to stability analysis for yield and maturity. Higher mean performance for yield (127.8 g /bulb) over general mean (111.4 g/bulb) with bi:1.09 and S²d of hybrid RH5 is an indicative of its most stable performance followed by other hybrids, RH2, RH6, RH10, RH17 and Virat (Table 1). There is only one hybrid i.e., RH9 which was found suitable for favourable environments and 2 hybrids, RH12 and RH13 for poor environments. If we consider their contribution towards yield increase over Virat only 3 hybrids viz., RH5, RH9 and RH13 gave more than 10% increase in yield (13.00%, 11.89% and 10.61% respectively) and hence, can be considered superior over commercial check (Virat). None of the white or yellow hybrids fulfilled the conditions of stability parameters and thus was of no concern for consideration. Incidentally, these 3 hybrids, RH5, RH9 and RH13 had considerable positive heterosis (26.50%, 47.70% and 17.00% respectively) with high specific combining ability (sca) effects of

	Mean performance of hybrids for yield/bulb (g)									
Sl. No.	Hybrid	KB 2020	KR 2020	WB 2020	SB 2021	SH 2021	Mean over seasons	bi	S ² d	Increase over Virat (%)
1	RH1	135.8	67.5	130.5	181.0	90.5	121.1	1.00	360.8	7.1
2	RH2	151.0	81.1	105.0	175.3	87.6	120.0	0.97	-27.4	6.1
3	RH3	145.5	53.8	86.0	183.3	91.6	112.0	1.22	131.7	0.9
4	RH4	160.8	60.0	103.5	179.8	89.9	118.8	1.19	34.9	5.0
5	RH5	163.0	76.5	121.5	185.3	92.6	127.8	1.09	53. 3	13.0
6	RH6	133.8	74.3	135.5	182.5	91.3	123.5	0.95	84.2	9.2
7	RH8	108.8	65.5	95.5	157.0	78.5	101.1	0.81	-57.8	-10.6
8	RH9	187.8	57.3	121.0	177.8	88.9	126.5	1.28	-72.4	11.9
9	RH10	140.0	76.3	102.0	174.3	87.1	115.9	0.96	-271.8	2.5
10	RH11	108.5	47.7	103.5	164.5	82.3	101.3	0.97	-21.5	-10.4
11	RH12	146.8	79.8	121.0	172.5	86.3	121.3	0.92	-360.5	7.2
12	RH13	150.5	93.3	140.5	160.8	80.4	125.1	0.73	-410.1	10.6
13	RH14	130.5	69.8	121.0	150.8	75.4	109.5	0.79	-329.2	-3.2
14	RH17	140.3	73.8	87.0	188.3	94.1	116.7	1.09	-44.9	3.2
15	WH1	120.3	53.0	96.0	157.5	78.8	101.1	0.95	-36.3	-10.6
16	WH3	159.3	74.8	71.0	162.8	81.4	109.8	1.02	364.9	-2.9
17	WH4	149.3	77.3	64.5	151.3	75.6	103.6	0.90	284.7	-8.4
18	WH5	119.0	46.8	92.5	156.0	78.0	98.5	0.99	-177.1	-12.9
19	WH7	151.0	35.0	80.5	146.5	73.3	97.3	1.16	37.7	-14.0
20	WH8	106.5	30.3	85.5	146.0	73.0	88.3	1.00	-68.8	-22.0
21	YH1	119.0	51.8	88.5	174.5	87.3	104.2	1.07	-4.7	-7.9
22	YH2	111.3	51.8	111.5	177.0	88.5	108.0	1.02	215.6	-4.5
23	YH3	137.5	69.0	91.5	148.3	74.1	104.1	0.85	-195.2	-8.0
24	YH4	115.0	53.4	84.5	166.0	83.0	100.4	1.00	-89.2	-11.2
25	Virat	127.5	61.3	123.5	168.8	84.4	113.1	0.95	49.5	0.00
26	Marshal	199.8	42.1	102.0	156.5	78.3	115.7	1.34	1038.1	2.3
27	Dhawal	188.8	50.7	98.0	184.5	92.3	122.8	1.41	381.0	8.6
	Mean	141.0	62.0	102.3	167.7	83.9	111.4	1.02	22.0	

TABLE 1: Stability analysis of onion hybrids for yield.

KB, *Kharif* Bengaluru. KR, *kharif* Ranebennur. WB, *Winter* Bengaluru. SB, *Summer* Bengaluru. SH, *Summer* Haveri. RH, Red Hybrid. WH, White Hybrid. YH, Yellow Hybrid.

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			Mean performance in d						
Sl. No.	Hybrid	KB 2020	WB 2020	SB 2021	SH 2021	Mean over seasons	bi	S ² d	Earliness over Virat (%)
1	RH1	89.0	79.5	88.5	83.5	85.1	0.53	0.2	-5.9
2	RH2	94.5	83.5	102.0	83.5	90.9	1.12	-3.1	0.4
3	RH3	93.0	82.0	101.0	81.0	89.3	1.17	-1.1	-1.4
4	RH4	94.0	75.0	86.5	81.5	84.3	0.78	33.6	-6.9
5	RH5	92.0	78.5	96.5	83.5	87.6	1.01	-3.8	-3.2
6	RH6	84.5	76.0	92.0	81.5	83.5	0.79	2.5	-7.7
7	RH8	94.5	83.5	101.0	84.0	90.8	1.06	-4.3	0.3
8	RH9	94.5	80.0	94.0	81.5	87.5	0.94	1.6	-3.3
9	RH10	92.5	83.5	89.5	81.0	86.6	0.56	8.5	-4.3
10	RH11	83.5	76.0	85.5	81.5	81.6	0.46	1.2	-9.8
11	RH12	98.5	82.0	97.5	81.5	89.9	1.11	7.7	-0.7
12	RH13	93.0	83.5	101.0	81.5	89.8	1.10	1.3	-0.8
13	RH14	88.0	82.0	99.5	81.5	87.8	0.98	8.7	-3.0
14	RH17	96.0	82.0	93.5	85.0	89.1	0.77	5.6	-1.5
15	WH1	100.5	85.0	107.0	81.5	93.5	1.48	5.5	3.3
16	WH3	95.0	85.0	107.0	86.5	93.4	1.22	4.0	3.2
17	WH4	94.0	85.0	107.0	87.0	93.3	1.18	8.0	3.0
18	WH5	95.0	83.5	105.0	86.0	92.4	1.20	-1.5	2.1
19	WH7	95.0	82.0	101.0	85.5	90.9	1.09	-5.5	0.4
20	WH8	100.5	85.0	103.5	84.5	93.4	1.23	-1.0	3.2
21	YH1	80.5	77.0	93.5	87.0	84.5	0.58	44.2	-6.6
22	YH2	87.5	77.0	89.5	81.5	83.9	0.70	-2.0	-7.3
23	YH3	94.0	77.0	102.0	84.5	89.4	1.34	-1.2	-1.2
24	YH4	94.0	77.0	102.0	83.5	89.1	1.37	-3.5	-1.5
25	Virat	96.5	83.5	95.5	86.5	90.5	0.77	1.3	0.0
26	Marshal	98.0	83.5	101.0	81.5	91.0	1.21	2.1	0.6
27	Dhawal	98.0	78.5	105.0	83.5	91.3	1.54	-6.9	0.8
	Mean	93.2	81.0	98.0	83.4	88.9	1.01	3.8	

TABLE 2: Stability analysis of onion hybrids for maturity.

KB, *Kharif* Bengaluru. WB, *Winter* Bengaluru. SB, *Summer* Bengaluru. SH, *Summer* Haveri. RH, Red Hybrid. WH, White Hybrid. YH, Yellow Hybrid.

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Hybrid

Female (190bp)

Male (180bp)

Fig.1: Molecular data indicating hybridity of onion hybrid RH5 at SSR marker ACM82



Fig.2: Bulbs of hybrid onion RH5.

20.30, 45.00 and 7.80 respectively thereby the hybrids are worth considering (unpublished data).

With regard to maturity, RH11 was earliest of about 82 d as against 89 d of general mean with bi of 0.46 adapting to poor environments (Table 2). However, RH5 was found to be the most stable hybrid that took 87.6 d for maturity as against Virat (90.5 d). Regression coefficient (bi) value of RH5 was 1.01, variance due to deviation from the regression (S²d) was -3.8. Five hybrids, RH1, RH6, RH9, RH11 and YH2 were found to be adapted to poor environments for maturity. Remaining 18 hybrids did not fulfill the stability model.

The molecular data for the stable hybrid, RH5 indicated double bands as against its 2 parents with single band at SSR marker, ACM82 (Fig.1).

DISCUSSION

Sustainability of crop yield over varying cultivation practices and changing climate is very important. The stability of yield and quality of variety or hybrid is appreciated in this context (Madalageri et al. 1998, Mohanty & Prusti 2001, Singh et al. 1995). Onion being cultivated in various states of India with varying soils, season and climatic zones with different open pollinated varieties; the productivity and quality of the marketable produce is very poor seeking for good hybrids that can suit to a wide range of cultivation with high yield and quality; and also reduces the problem of seed production of many varieties to be maintained at each level of purity as being a highly out crossing crop. The earlier attempts to popularize onion hybrids in India were not

successful because of the introduced parentages (long day types) from other countries that did not suit to our climate. The present attempt used the material developed using landraces of our country and developed hybrids that were tested in diverse conditions and came out with the hybrids that are found suitable in their performance. Thus hybrid, RH5 was stable for its yield and early maturity as compared to variety, Virat (Fig.2). The hybrid vigour or hybridity of RH5 was confirmed by marker analysis and its superiority by about 13% increase in yield and 3% earliness is hoped to catch the attention of onion farmers. Yet there are hybrids like RH9 and RH13 that can do well in irrigated, better maintained conditions and rainfed, low input farming respectively which need to be considered wherever necessary. Similar opinion was expressed by Khar et al. (2007), Sidhu et al. (1998), Singh et al. (1984) and Varalakshmi & Reddy (1998).

From the results of the present study it can be concluded that, RH5 was the most stable hybrid for obtaining higher yield and early maturity at any environments/locations and may help the onion growing farmers of the country and state.

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REFERENCES

- ANNONYMOUS 2019 National horticulture data base New Delhi
- EBERHART S A & RUSSELL W A 1966 Stability parameters for comparing varieties *Crop Sci* **6** 36–40

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- JIAN-FENG LI, LILI & JEN SHEEL 2010 Protocol: a rapid and economical procedure for purification of plasmid or plant DNA with diverse applications in plant biology *Plant Methods* **6**1
- KHAR AA, ASHA DEVI V, MAHAJAN & LAWANDE K E 2007 Stability analysis of some elite onion lines in late *kharif* season *Indian J Hort* **64** 415–419
- MADALAGERI B B, BOJAPPA K M & SOMASEKHER C 1998 Onion breeding for higher solids genetic variation heritability and stability *Indian J Hort* **43** 248–252
- MOHANTY B K & PRUSTI A M 2001 Genotype × environment interaction and stability analysis in *kharif* onion *Veg Sci* **28** 17–21

- PUJARA D S, PURUSHOTHAMA M G & MADALAGERI B B 2017 Identification and development of cytoplasmic male sterile maintainer and restorer lines in short day Indian onions using molecular markers J Cytol Genet 18 (NS) 67–73
- SIDHU A S, CHADHA M L, SINGH S & THAKAR M R 1988 Phenotypic stability in onion (*Allium cepa*) Indian J Agric Sci **58** 481–482
- SINGH J, PANDEY U C & RANA M K 1995 Stability parameters for desirable traits in onion (*Allium cepa* L) cultivars *Haryana J Hort Sci* 24 60–64
- SINGH P S, YADAV B S & NARSINGHANI V G 1984 Stability of yield components in pea *J Agric Sci* 54 608–612

RESEARCH ARTICLE

CHROMOSOME STUDIES ON TWO SPECIES OF *NEUROTHEMIS* (ANISOPTERA:ODONATA) FROM HIMACHAL PRADESH

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SUMMARY The present study concerns karyomorphology and meiotic analysis in *Neurothemis fulvia* and meiosis in *N. tullia*. In *N. fulvia*, the diploid chromosome number was 25 with a pair of **m** chromosomes. The chromosomes were holocentric. The chromosome length ranges from 0.45 μ m to 2.91 μ m and the total of the diploid complement was 45.89 μ m. The actual length and relative length of X chromosome were 1.12 μ m and 2.42 μ m respectively. In both species, the haploid chromosome number was 13.

Keywords: Chromosomes, Neurothemis, holocentric, total complement length, karyotype.

INTRODUCTION

Approximately 7000 species of odonates lie around the world over (Kalkman et al.2008). Libellulidae is the richest and most widely distributed family in the suborder Anisoptera. This family represented by 1035 species under 144 genera from all over the world and about 91 species under 40 genera from India (Subramanian & Babu, 2017). Cytogenitically, this family is well studied as compared to other families with data available for approximately 270 species (Walia et al. 2011). The modal chromosome number of the family is 2n = 25 including a pair of m chromosomes which is found in about 90% of the species. The chromosomes are holocentric and most frequent sex determination system is XX/XO type. Cytogenetic studies of this family were done earlier by various workers (Asana & Makino 1935, Dasgupta 1957, Kiauta 1975, Sandhu & Walia 1994, Thomas & Prasad 1981, 1986, Walia 2008, Walia & Sandhu 2002).

A peculiar feature observed in dragonflies is the presence of minute **m** chromosomes. These are the smallest chromosomes among autosomes formed as a result of fragmentation and diminution of chromosomes and are present nearly in about 80% of the species.

Babu (2014) documented 125 species of odonates from Himachal Pradesh. So far, no detailed information is available on the chromosomes of these odonates. Due to lack of data on the chromosomes of *Neurothemis fulvia* and *N. tullia* from Himachal Pradesh, it was considered desirable to study the chromosomes of these species.

MATERIALS AND METHODS

For chromosomal preparations, live male specimens of *N. fulvia* and *N. tullia* were collected from Sirmaur (30.4453°N latitude and 77.6021°E longitude and altitude 389 m above sea level)) and Kangra (32.2817°N latitude and 75.8512°E

longitude and altitude 489 m above sea level) districts of Himachal Pradesh during months of June-September. Identification of individuals was done on the basis of keys provided by Fraser (1936). Germinal tissue was dissected out in normal saline solution after anaesthetizing the individuals with chloroform and pretreated with 0.7% sodium citrate solution for 30 min at room temperature. The material was fixed in 1:3 acetic acid-ethanol solution for 25-30 min and squashed in 45% acetic acid by gentle tapping and pressing the slides with cover slips in between the folds of blotting paper. Cover slips were dislodged off from the slides with a sudden jerk. The slides and cover slips were air dried for 2 or 3 d in a dust free chamber.

After drying, staining was done in 2% Giemsa for 20 min. The slides were then mounted in DPX and observed under binocular research microscope. Well spread chromosome complements were selected for photomicrography and chromosomal measure-ments. Lengths of chromosomes were measured using ocular micrometer and total complement length was calculated. The relative lengths (percentages of total complement length) were calculated and idiogram was constructed based on relative lengths. Karyotype was prepared from photomicrographic print.

OBSERVATIONS

In the present study, chromosomes of two species of dragonflies have been studied.

N. fulvia

It is a medium-sized reddish brown dragonfly having reddish brown wings with an irregular, triangular, transparent area at the tip. The diploid chromosome number was found to be 25 with a pair of **m** chromosomes (Fig. 1). The mean actual length of chromosomes ranged from 0.45 μ m \pm

0.04 S.E. to 2.91 μ m \pm 0.08 S.E. and the total complement length was 45.89 μ m \pm 0.90 S.E. The mean relative length of chromosomes ranged from 0.97 \pm 0.08 S.E. to 6.33 \pm 0.13 S.E. The mean actual length and the mean relative length of X chromosome were 1.12 μ m \pm 0.06 S.E. and 2.42 \pm 0.09 S.E. Karyotype and idiogram showed gradual decrease in chromosome lengths with **m** chromosome pair as the smallest element of the complement (Figs 2, 3).

For meiotic studies, chromosomes were observed at spermatogonial metaphase and different meiotic stages i.e., pachytene, diakinesis, metaphase I, prophase II and metaphase II. During pachytene, the chromosomes appeared as network of entangled threads (Fig. 4). At diakinesis, 13 elements were visible which include **m** bivalent and univalent X chromosome. Bivalents were cross shaped having single interstitial chiasma (Fig. 5). As diakinesis advanced chiasma localized to terminal position (Fig. 6). At metaphase I, chromosomes showed extreme condensation (Fig. 7). At prophase II, chromosomes attained characteristic ɛ-shape (Fig. 8). At metaphase II, 13 elements were observed with X and **m** chromosome at the peripheral position and **m** chromosome was lightly stained (Fig. 9).

N. tullia

It is a black dragonfly with a pale yellow, middorsal carina of thorax. Wings are hyaline for apical half and black for basal half, which is bordered by a milky white patches towards the tips. Chromosomes were observed at pachytene and diakinesis. The haploid chromosome number in this species was found to be 13 including **m** bivalent.

During pachytene, the chromosomes appeared as distinct threads showing cross with each other

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Figs 1–11: 1–9. *N. fulvia*. 1–3. Karyotype. 1. Spermatogonial metaphase (2n=25m). 2. Karyotype. 3. Idiogram. 4–9. Meiosis. 4. Pachytene. 5. Early diakinesis. 6. Diakinesis. 7. Metaphase I. 8. Prophase II. 9. Metaphase II. 10,11. *N. tullia*. 10. Pachytene. 11. Diakinesis (n=13m). (m, m chromosome, X, X chromosome)

at certain places (Fig. 10). At diakinesis, 13 elements were visible out of which 12 were bivalents and a single univalent X chromosome. Bivalents showed rectangular shape due to the presence of single chiasma in each while X chromosome was round in shape. The **m** bivalent was the smallest autosomal bivalent (Fig. 11).

DISCUSSION

In the two species of *Neurothemis* studied here, the diploid chromosome number was 2n = 25 with a pair of **m** chromosomes. Earlier, 4 species namely, N. fulvia, N. intermedia, N. terminata and N. tullia of this genus had been described cytogenetically from different localities by various workers (Kiauta 1969, 1974, 1975, Kiauta & Kiauta 1980, Kiauta & Kiauta 1983). No data were available on the chromosomes of N. fulvia from India yet. However, present investigation revealed 2n = 25m in *N. fulvia* with XO sex determination system which was in agreement with the reports of Kiauta (1974, 1975) who collected samples from Nepal. During the present investigation, other species studied was *N. tullia*, which also showed n = 13 m with XO sex determination system, which suggested diploid chromosome number as 2n = 25m. This chromosome number was in agreement with earlier findings of Handa et al. (1984) and Sandhu & Walia (1994). Earlier reports in N. tullia showed high variations with respect to chromosome number and sex determination system. Ray Chaudhuri & Dasgupta (1949) and Tyagi (1978) reported 2n = 28 with neo-XY sex determining system. Thomas & Prasad (1986) reported 2n = 27with XO type sex determination system. This increase or decrease in chromosome number might be attributed to the fission or fusion of the chromosomes, thereby exhibiting chromosome

variation in different populations.

REFERENCES

- ASANA J J & MAKINO S 1935 A comparative study of the chromosomes in the Indian dragonflies *J Fac Sci Hokkaido Imp Univ Ser* **4** 67–86
- BABU R 2014 Diversity of dragonflies (Odonata) in Himachal Pradesh India *Agrion* **18**41–47
- DASGUPTA J 1957 Cytological studies of some Indian dragonflies II A study of the chromosomes during meiosis in thirty species of Indian Odonata (Insecta) *Proc Zool Soc Calcutta* **10**1–65
- FRASER F C 1936 Fauna of British India including Ceylon and Burma Odonata Vol III Taylor & Francis London
- HANDA S M, MITTAL O P & BATRA H N 1984 Chromosomes in ten species of dragonflies (Anisoptera Odonata) Res Bull Punjab Univ Sci 35 65–75
- KALKMAN V J, CLAUSNITZER V, DIJKSTRA K D B, ORR A G, PAULSON D R & VAN TOL J 2008 Global diversity of dragonflies (Odonata) in freshwater *Hydrobiologia* 595 351–363
- KIAUTA B 1969 Sex chromosomes and sex determining mechanisms in Odonata with a review of the cytological conditions in the family Gomphidae and references to the karyotypic evolution in the order *Genetica* 40 127–157
- KIAUTA B 1974 Introduction to insect cytotaxonomy Lectures delivered at the Tribhuvan University Kathmandu Vol 1 Nepal Research Center Kathmandu pp 81
- KIAUTA B 1975 *Cytotaxonomy of dragonflies with special reference to the Nepalese fauna* Nepal Research Centre Kathmandu
- KIAUTA B & KIAUTA M A J E 1980 On a small collection of dragonfly karyotypes from the Philippines Odonatologica 9 237–245
- KIAUTA B & KIAUTA M A J E 1983 The chromosome numbers of some Odonata from Thailand *Notul Odonatol* 227–28
- RAY CHAUDHURI S P & DASGUPTA J 1949 Cytological studies on the Indian dragonflies I Structure and behaviour of chromosomes in six species of dragonflies (Odonata) *Proceeding of the Zoological Society of*

Bengal 2 81-93

- SANDHU R & WALIA G K 1994 Chromosomal studies of three species of libellulids (Anisoptera Odonata) La Kromosoma II 75–76 2599–2604
- SUBRAMANIAN K A & BABU R 2017 A Checklist of Odonata (Insecta) of India Version 3 Zoological Survey of India Kolkata
- THOMAS K I & PRASAD R 1981 The chromosomes of five Indian dragonflies (Odonata) *Perspect Cytol Genet* **3** 629–632
- THOMAS K I & PRASAD R 1986 A study of the germinal chromosomes and C-band patterns in four Indian dragon-

flies (Odonata) Perspect Cytol Genet 5 125-131

- TYAGI B K 1978 The chromosome numbers and sex determining mechanisms newly recorded in thirteen Indian dragonflies (Odonata) *Chrom Inf Serv* **25** 5–7
- WALIA G K 2008 Comparative cytological data on twenty six species of family Libellulidae (Anisoptera Odonata) *Fraseria* 7 77–122
- WALIA G K & SANDHU R 2002 Chromosomal data on seven species of genus Orthetrum (Libellulidae Anisoptera Odonata) Bionature 22 7–12
- WALIA G K, KAUR H & KAUR J 2011 Karyotypic variations in the chromosome complement of *Pantala flavescens* (Fabricius) of the family Libellulidae (Anisoptera Odonata) *Cytologia* **76** 301–307