VERSITA

Central European Journal of Biology
DOI: 10.2478/s11535-006-0022-6
Research article
CEJB 1(3) 2006 386–398

Features of crossing-over in virus-infected tomato

Gheorghe I. Chiriac, Larisa I. Andronic*, Valeriu V. Bujoreanu, Liliana I. Marii

Institute of Genetics,
Academy of Sciences of Moldova,
Chisinau, MD 2002, Republic Moldova

Received 18 April 2006; accepted 28 May 2006

Abstract: The evidence of increased crossing over rate in tomato hybrids infected with TAV (Tomato aspermy virus), PVX (Potato virus X), TMV (Tobacco mosaic virus), TMV+PVX indicates the recombinogenic effect of viral infection. Cytological studies of the early diakinesis in healthy and virus-infected tomato revealed significant changes in chiasma number and position. The most significant changes were established for bivalents with two interstitial chiasmata and with one terminal and one interstitial. The data obtained indicate redistribution of the chiasmata position and induction of additional exchanges. The virus-induced recombination is segment-specific and depends on the host plant genotype, virus infection and the interaction between them.

Keywords: Chiasma, crossing-over, marked segment, recombinogenesis, tomato, virus infection

1 Introduction

Considerable evidence has accumulated to date suggesting the possibility of increasing recombination frequency and spectrum through the use of chemical, physical and biological factors and by the interaction of these with environmental conditions [1, 2]. Inducers of genetic variability include phytopathogenic viruses which, according to some authors, can be used to produce recombinants [3–6]. At the same time, studies addressing specific features of crossing-over modification in virus-infected plants are few and far between.

The modification process of chromatid segment exchange within homologous chromosomes can be understood by evaluating chiasma distribution in meiotic prophase I and recombination frequency in marked segments. Chiasmata are considered to be the cy-

* E-mail: andronic17@yahoo.com

tological expression of crossing-over and, therefore, their number and distribution reflect the location and degree of chromosome pairing [7]. Cytogenetically, bivalent configurations at different stages of meiosis permit evaluation of chromosome pairing. According to some authors [8–10] the proportions of various types of bivalents can be used to assess modifications in genetic variability release. Changes in chiasma distribution pattern during diplotene-diakinesis can offer a sure method for estimating the effect of genetic factors and environment on recombination frequency and spectrum [2]. The available techniques can be used to detect changes in recombination rates of virus-infected plants. The objective of the present work is to study crossing-over in tomato plants and the way it is affected by viral infection, host plant genotype, experimental environmental conditions and by the interaction of these factors.

2 Experimental procedures

2.1 Biological material

The plant material used in this study were tomato (*Lycopersicon esculentum* Mill, 2n=24) cultivars Licurič, Nistru, Prizer and Fakel, tomato F_1 intracultivars hybrids Nota × Krasnoârskij rannij, Novičok × Kolokolčik, Mo 393 × Victorina as well as F_1 hybrids heterozygous for recessive linked genes d–aw (chromosome 2), ful-e (chromosome 4), c-m-2 (chromosome 6) and hl-a (chromosome 11). The gene symbols and localization are as in Atherton and Rudich [11]. The mutant lines M$_\circ$393 (*c-m-2*/++), M$_\circ$500 (*c-m-2*/++; *d-aw*/++), 504 (*d-aw*/++) and M$_\circ$628 (*ful-e*/++; *hl-a*/++) [12], were used as parental stocks to produce F_1 hybrids heterozygous for marker analysis. Plants were grown in pots in the greenhouse following a standard technique.

2.2 Virological analysis

Control plants were mock inoculated with healthy tomato extract. Experimental plants at the stage of 6-8 leaves were infected with Tobacco mosaic virus (TMV), Potato virus × (PVX), Tomato aspermy virus (TAV) and the TMV+PVX combination. Further work involved material collected from plants showing external disease symptoms and found to contain virions in flower buds undergoing meiosis. Controls were healthy plants which responded negatively to virological test at the time of collecting flower buds undergoing meiosis or fruit from F_1 plants prior to removal of seeds from them. In the case of TMV infection, seeds of all treatments were exposed to a temperature of 70 °C for 72 hours.

Presence of virions in infected plants and their absence from healthy plants was established through ELISA [13] and ISEM [14] tests. Immunological analysis was carried out using a monovalent serum and diagnostic kits.

2.3 Cytological analysis

For cytogenetic analysis, flower buds undergoing meiosis were fixed following the method of Clarke [15]. Chiasma frequency was measured based on the studies of temporary preparations obtained by acetocarmine staining [15] and studied under light microscope BIOLAR. Bivalents were grouped by configuration according to chiasma number and positions during diakinesis [16]: 1 interstitial (I), 2 interstitial (II), 1 terminal (T), 1 interstitial + 1 terminal (I+T), 1 terminal + 2 interstitial (T+II), 2 terminal (TT), 2 terminal + 1 interstitial (TT+I), and univalents (0). Total number of interstitial chiasmata (SI), terminal chiasmta (ST) and the overall number of chiasmata (S) per pollen mother cell (PMC) were assessed simultaneously.

2.4 Statistical analysis

The experiments were conducted during 1984-2002. The statistical processing of data was carried out using analysis of variance [17]. The contribution of variation sources was computed following the method of Dospehov [18] based on the ANOVA test results. For statistical analysis we used the software Statgraphics Plus for Windows (version 2.1; Microsoft Corp., Redmond, WA, USA).

3 Results

3.1 Cytological analysis

Diakinesis is the best cell stage for estimation of chiasmata frequency, because pseudochiasmata resulting from the twisting of bivalents cease to be observed in this stage and pseudoterminalization due to contraction of bivalents is less prominent than in metaphase I [19]. The cytological studies of the early diakinesis established typical meiotic bivalent figures in healthy and virus infected tomatoes (7 cultivars and hybrids) (Figures 1a – 1e).

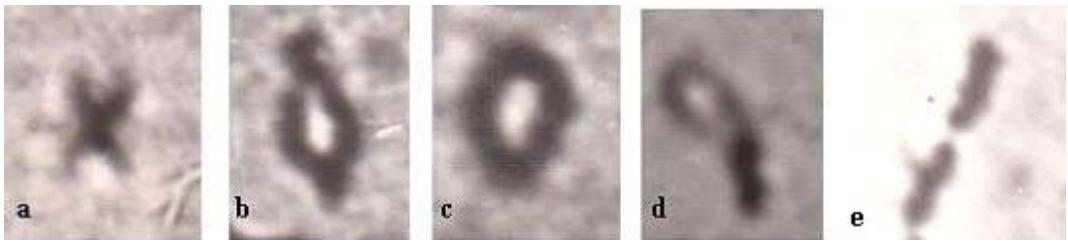

Fig. 1 Bivalent configuration according chiasma position during diakineses: 1I (a), II (b), TT (c), T+I (d), T (e) (× 6500).

Meiotic spreads analysis at diakinesis showed in tomato pollen mother cells the normal bivalent number (n = 12) (Figures 2a and 2b). Results show that the difference between treatments consist only in numerical variations of the bivalent types.

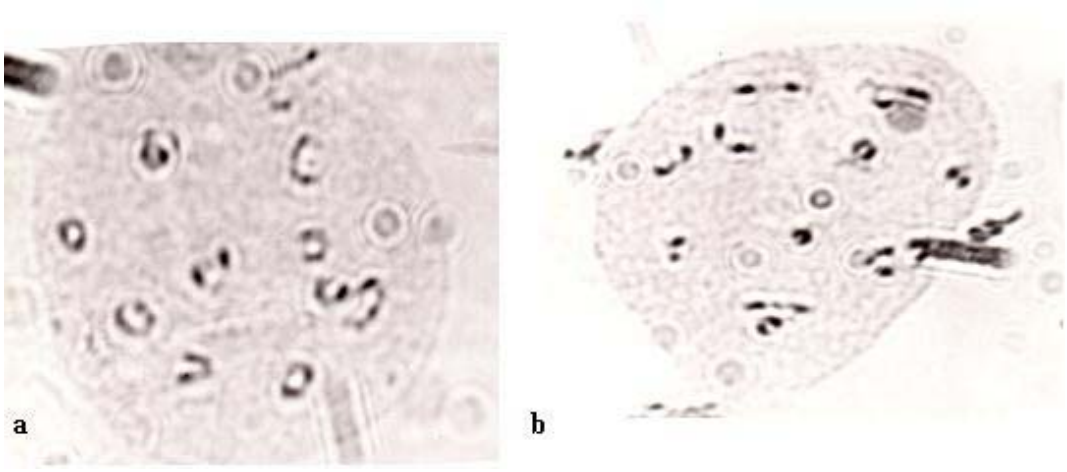

Fig. 2 Tomato pollen mother cells of healthy (a) and TAV infected (b) Fachel cultivar at diakinesis (staining with acetocarmine) (× 2500).

The experiments conducted have demonstrated that tomato plants infected with TAV and the TMV + PVX combination responded by increasing the total chiasma number per PMC (Table 1). Significant differences have been observed for all host-virus combinations. The greatest changes recorded were those in the number of interstitial chiasmata.

Bivalent type	Control	TMV + PVX	TAV
I	0.67±0.02	0.83±0.03***	0.83±0.04***
II	0.40±0.03	0.77±0.04***	0.67±0.05***
T	6.73±0.11	4.70±0.18***	5.42±0.21***
T+I	1.64±0.05	2.47±0.09***	2.33±0.10***
T+II	0.05±0.01	0.07±0.01***	0.02±0.02***
TT	1.80±0.09	1.70±0.15**	1.24±0.17***
TT+I	0.74±0.06	1.13±0.09***	1.30±0.11***
0	0.11±0.03	0.34±0.05***	0.32±0.05***
SI	4.03±0.09	6.39±0.15***	5.29±0.17***
ST	13.11±0.13	12.88±0.21***	12.46±0.25***
S	17.07±0.12	19.06±0.16***	17.59±0.23***

; * - significant at P ≤ 0.01; 0.001

Table 1 Distribution of bivalent types per PMC in healthy and TAV or TMV+PVX infected tomato genotypes.

The data presented in Table 2 suggest that variation in interstitial chiasmata per PMC is due to viral infection (71.88%), the genotype (6.82%) and the interaction of these (11.26%).

Analysis of variance revealed a significant effect of the genotype and viral infection on the majority of bivalents, as well as a very strong interaction of these factors. The number of bivalents with one interstitial chiasma and two terminal chiasmata was not

Bivalent type	Variation source	Df	F-ratio	Contribution of variation source (%)
I	Genotype (A)	8	12.24***	33.40
	Virus (B)	1	76.42***	25.56
	Interaction A × B	10	14.38***	28.76
II	Genotype (A)	8	23.98***	20.70
	Virus (B)	1	407.59***	50.86
	Interaction A × B	10	20.74***	23.94
T	Genotype (A)	8	12.88***	6.68
	Virus (B)	1	600.97***	65.42
	Interaction A × B	10	47.17***	24.46
T+I	Genotype (A)	8	14.62***	18.46
	Virus (B)	1	331.70***	55.69
	Interaction A × B	10	13.07***	18.46
T+II	Genotype (A)	8	0.67***	42.32
	Virus (B)	1	0.74	0.46
	Interaction A × B	10	7.08***	34.81
TT	Genotype (A)	8	38.89***	19.93
	Virus (B)	1	131.10***	8.40
	Interaction A × B	10	135.35***	69.37
TT+I	Genotype (A)	8	14.45***	37.82
	Virus (B)	1	79.08***	25.87
	Interaction A × B	10	9.38***	24.54
0	Genotype (A)	8	0.97 0.72	9.99
	Virus (B)	1	22.79***	35.97
	Interaction A × B	10	0.72	7.35
SI	Genotype (A)	8	3.06***	6.82
	Virus (B)	1	257.95***	71.88
	Interaction A × B	10	5.05***	11.26
ST	Genotype (A)	8	25.31***	27.08
	Virus (B)	1	54.78***	7.33
	Interaction A × B	10	56.80***	60.75
S	Genotype (A)	8	16.34***	18.01
	Virus (B)	1	345.31***	47.57
	Interaction A × B	10	26.73***	29.45

*** - significant at $P \leq 0.001$

Table 2 Analysis of variance by bivalent type in virus-infected tomato plants.

changed by the viruses studied. For the T+II configuration, a compensation effect was observed: the number of bivalents with 1 interstitial chiasma and 2 terminal chiasmata increased following infection of tomato plants with the TMV+PVX combination and decreased upon infection with TAV. The evaluation of percent contribution of variation sources has revealed that, upon infection of tomato plants with TAV or TMV+PVX, the number of bivalents with 1 terminal chiasma varied the most, followed by bivalents with

1 terminal chiasma + 1 interstitial chiasma and, finally, bivalents with 2 interstitial chiasmata. The number of bivalents of the first type decreased whereas that of the second and third type increased, suggesting that the interstitial chiasma frequency in infected tomato plants increases due to the greater number of bivalents with 1 or 2 interstitial chiasmata.

The distribution of bivalent types into groups according to the correlations between them was carried out with the aim of ascertaining whether there is a direct relationship between the total number of chiasmata and the particular bivalent type. Based on the dendrograms presented in Figure 3, the pattern of the within-cluster distribution of bivalents in treatments was found to be different from that in the control.

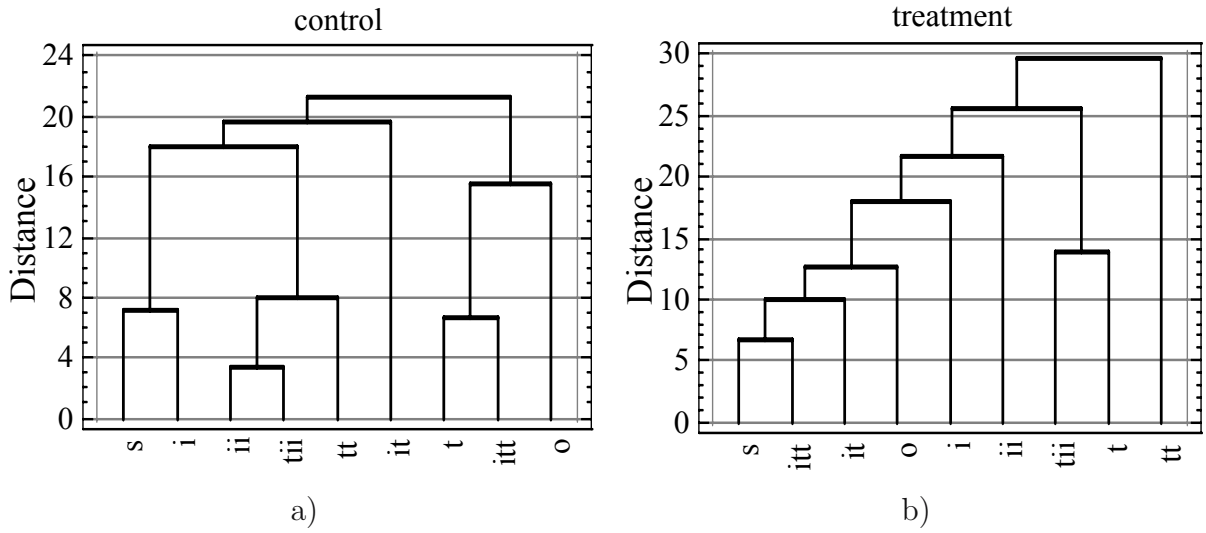

Fig. 3 Distribution of bivalent types by degree of correlation in healthy and virus-infected tomato plants ('distance' on Y is expressed in Squared Euclidian).

Analysis of the 4 virus-genotype combinations by the Nearest Neighbor Method according to the degree of correlation showed redistribution of bivalents within clusters by both the total chiasma number and the bivalent type.

3.2 Analysis of crossing-over in marked segments

Recombination frequency estimated for tomato plants inoculated with three viruses and a mixed infection reveals recombinogenic effect of virus infection which varies according to the host and pathogen genotype, chromosome segment under study, experimental environmental conditions (replications per year) and interaction of these factors (Tables 3, 4 and 5).

All the viruses used induced significant changes in the frequency of recombination in some of the segments studied, but not in all segments and not in all genotypes used in the experiments. For example, the Mo 393 × Mo 504 hybrid responded to TMV-infection by a significant decrease in the rate of recombination between markers *d* and *aw*. The level of recombination in the *c-m-2* segment of this TMV-infected hybrid remaining unchanged.

Combination	Virus	Control	Treatment	% of the control
		rf_{c-m-2}		
Mo 393 × Fakel	TAV	28.11±1.05	26.92±0.92	95.77
Mo 500 × Mo Peron	TAV	24.16±1.16	28.72±2.48*	118.87
Mo 500 × Mobaci	TAV	23.06±1.23	22.24±2.52	96.44
Mo 500 × Krasnoârskij rannij	TMV+PVX	26.58±1.22	27.49±0.94	103.42
Mo 500 × Mobaci	PVX	23.06±1.23	20.66±2.68	89.59
Mo 500 × Mo Peron	PVX	24.16±1.16	23.60±1.97	97.68
Mo 393 × Mo 504	TMV	30.27±2.15	27.03±2.19	89.35
Mo 393 × Mobaci	TMV	21.33±1.09	21.92±1.43	102.77
		rf_{d-aw}		
Mo 393 × Mo 504	TMV	13.51±2.51	7.91±1.84*	58.55
Nistru × Mo 500	TMV	7.59±1.01	5.80±2.18	76.42
Mo 500 × Krasnoârskij rannij	TMV + PVX	10.39±1.11	13.75±0.86**	132.34
Mo 500 × Mobaci	PVX	8.23±0.72	8.68±1.71	105.47
Mo 500 × Mo Peron	PVX	7.34±0.81	10.87±0.93***	148.09
Mo 500 × Mo Peron	TAV	7.34±0.81	8.07±1.20	109.94
Mo 500 × Mobaci	TAV	8.23±0.72	12.61±2.57*	153.22

*; **; *** - significant at P ≤ 0.05; 0.01; 0.001

Table 3 Frequency of crossing-over (%) in tomato plants as a function of the virus-genotype combination.

The same effects were observed in treatments in which tomato plants were infected with TAV, PVX and TMV+PVX. Thus, the F_1 hybrid Mo 500 × Krasnoârskij rannij infected with the TMV+PVX combination showed significant changes in rf in the *d-aw* segment but not in the *c-m-2* segment. In case of the Fakel × Mo 628 hybrid infected with TAV, significant differences have been noted for rf_{hl-a}, whereas rf_{ful-e} was the same as in healthy plants. The level of rf_{d-aw} decreased by 23.42-41.45% when genotypes were infected with TMV and increased by 53.22% and 48.09%, when these genotypes were infected with TAV or TMV+PVX, respectively.

It is worth noting that where there is no significant difference in recombination frequency, the recombinogenic effect of the factor examined cannot be ruled out. Analysis of variance indicates that rf_{c-m-2} was significantly affected by both the genotype and viral infection. The percent contribution of different sources of variation to the rf modification in the *c-m*-2 segment estimated for 8 virus-genotype combinations was as follows: virus infection – 31.19%, genotype – 36.06%, virus-genotype interaction – 24.68% (Table 4).

In our experiments the rate of recombinants in the segments studied in healthy plants varied from one year to the next, the differences being statistically significant in some cases. Thus, rf_{d-aw} varied from 9.01±0.43 to 12.75±0.85, and rf_{hl-a}) from 17.38±2.07 to 22.28±2.80 (Table 5). This suggests that crossing-over rate variations were due to

Variation source	Contribution of variation source (%)	Df	F-ratio
	rf_{d-aw}		
Virus (A)	46.70	2	32.74***
Genotype (B)	18.29	2	12.82***
A × B	22.17	4	7.77***
	rf_{c-m-2}		
Virus (A)	31.19	2	34.76***
Genotype (B)	36.06	2	40.19***
A × B	24.68	4	13.75***
	rf_{d-aw}		
Virus (A)	51.35	1	104.70***
Environment (C)	42.38	2	43.21***
A × C	0.38	2	0.38
	rf_{c-m-2}		
Virus (A)	15.94	1	108.47***
Environment (C)	81.50	2	277.00***
A × C	0.78	2	2.65
	rf_{hl-a}		
Virus (A)	55.00	1	116.94***
Environment (C)	32.75	2	34.80***
A × C	6.58	2	6.99**

; * - significant at P ≤ 0.01; 0.001

Table 4 Analysis of variance for rf in tomato genotypes heterozygous for *d*, *aw*, *c*, *m-2*, *hl* and *a* genes and infected with TMV or TAV, PVX, TMV+PVX.

different environmental conditions to which F_1 hybrids were exposed during their growth. At the same time, infection of plants with TMV affected the frequency of recombination between markers such that the differences between treatments and the control expressed in percentages were approximately the same for 3 experiments in 3 different years (Table 5).

Analysis of variance has shown that rf_{d-aw} and rf_{c-m-2} were strongly affected by the genotype and environmental conditions, but not by the interaction between these factors, during the years of the experiments. For segment *hl-a* on chromosome 11, the effect of environmental conditions or virus was statistically significant at 1%, and the effect of the interaction of these factors at the significance level of 0.1%. Experiments were conducted in different years on non-infected Mo 393 × Mo 504 hybrid (replications 2 and 3 compared to replication 1).

Combination	Treatment No.	Control	Treatment	% of the control
		rf_{d-aw}		
Mo 393 × Mo 504	1	12.75±0.85	9.71±0.50***	76.16
Mo 393 × Mo 504	2	9.01±0.43^{++}	6.90±0.22***	76.58
Mo 393 × Mo 504	3	11.17±0.77^{+}	8.37±0.57***	74.93
		rf_{c-m-2}		
Mo 393 × Mo 504	1	30.68±2.03	27.86±2.15	90.81
Mo 393 × Mo 504	2	26.05±2.05	24.35±2.89	93.47
Mo 393 × Mo 504	3	31.06±1.94	29.98±2.14	96.52
		rf_{hl-a}		
Mo 628 × Barnaul conservny	1	22.28±2.80	18.56±2.31	83.30
Mo 628 × Barnaul conservny	2	21.51±2.33	15.91±2.46*	73.96
Mo 628 × Barnaul conservny	3	17.38±2.07	12.81±1.39*	73.70

*; *** - significant at P ≤ 0.05; 0.001

Table 5 Recombination frequency in some marked segments of TMV-infected tomato plants in replications over years.

4 Discussion

Chiasma distribution and frequency are two important parameters to examine in crossing-over analyses [19]. We observed significant modifications in the positions of terminal and interstitial chiasmata in tomato plants infected with TAV or TMV+PVX. According to early investigations [20] the interstitial chiasmata can move to a terminal position. Apart from the chiasmata terminalization theory, the viewpoint held by the most authors is that chiasma localization reflects the position of exchange [21, 22]. Analyses of labelling patterns in autoradiographs in X_2-labelled diplotene bivalents showed that chiasma terminalization is not expected to produce gaps [23]. The theory of chiasmata terminalization was refuted by BrdU labeling techniques [24, 25]. So-called terminal chiasma were best interpreted by an achiasmatic terminal association [26] resulting from the telomerenuclear membrane association [19]. A previous study demonstrates that interstitial chiasmata do not move [16], which implies that a terminal association cannot be formed by chiasma terminalization. Generally, chiasma terminalization results in an overall reduction in number of chiasmata in bivalents, and in chiasmata being concentrated at or near the bivalent ends. We did not observe these phenomena. Moreover, the terminalization coefficient in healthy and infected plants was similar and varied from 0.68 in TMV+PVX-infected plants to 0.77 in healthy plants. Variation in chiasma frequency and localization is genetically controlled [27], and chiasma distribution can be used in specimen studies [28].

Our results suggest that, following infection of the genotypes under study with TAV or TMV+PVX, redistribution and induction of new exchanges of chromatid segments

occurred. Similar effects were observed in plants carrying supernumerary B chromosomes which induce changes in pairing of A chromosomes and in frequency and distribution of chiasmata within chromosomes [29, 30]. According to Bell and Burt [31], exogenous DNA induces chiasma redistribution as a result of an increase in the number of interstitial chiasmata, and a concurrent decrease in the number of terminal chismata. Such redistribution is significant because it increases recombination of genes localized in the same linkage groups. It should be noted that a similar chiasma distribution was observed in our experiments following the infection of tomato plants with RNA viruses which are regarded also as parasites at the genetic level [32]. The virus-infected tomato plants also exhibited modification of the rate and spectrum of recombination [6].

Changes in the number of recombinants in segregating populations following the infection of F_1 hybrids with viruses were also demonstrated through estimation of crossing-over in marked segments. Significant deviations from the control in the number of recombinants between genetic markers localized on chromosomes 2, 6 and 11 were observed. This could be due to crossing-over rate modification or to phenotypic expression of markers in F_2 populations. Sorghum (*Sorghum bicolor L.*, Moench) plants infected with Sugarcane mosaic virus showed distortion of segregation for seedling color and ligule expression in segregating progeny [33]. In our experiments, significant deviations from the 3:1 segregation ratio were not observed for any analyzed markers. These results indicate that the differences between treatments and the control in the number of recombinants are due to modifications of recombination frequency in marked segments in the virus-infected F_1 hybrids. These observations are corroborated by changes in the number and spectrum of chiasmata per PMC in virus-infected plants. Thus, TMV, TAV, PVX and the TMV+PVX combination can be regarded as inducers of recombination in tomato.

Our results are consistent with the induced recombinogenesis peculiarities described in the literature. It has been shown that a single factor can bring about diametrically opposite changes in different segments, and a single segment responds in different ways to different factors [2]. Similar results were obtained when tomato plants were infected with TMV, TAV, PVX and TMV+PVX. Upon infection with viruses, some segments responded by changes in the number of recombinants, whereas other segments appeared to be more inactive. Among the former is the *d-aw* segment on chromosome 2. Crossing-over rate was considerably reduced in hybrids infected with TMV and increased in those infected with PVX, TAV and TMV+PVX. At the same time, no changes in recombination frequency in the *ful-e* segment were observed in TMV- or TAV-infected plants, and significant deviations of rf_{c-m-2} were noted in one of the eight treatments. This suggests that modification of crossing-over rate in virus-infected tomato plants depends on the segment under study, and on the genotype of the host plant and of the virus applied.

The frequency and spectrum of recombinants are also affected by the environmental conditions to which F_1 hybrids are exposed during their growth. Factors such as temperature, moisture deficit and excess of nutrients can bring about modifications in the genetic structure of segregating populations [34]. The results from experiments conducted in different years on TMV-infected F_1 hybrids are consistent with the above results. In

the same experiments, the interaction of a virus and environmental conditions was found to affect the recombination frequency in the segments under study to a lesser extent than either of the factors acting singly. This is significant from the standpoint of using phytopathogenic viruses as inducers of genetic variability. Of special interest are those recombinogenic factors whose activities are not greatly influenced by other factors [2].

Deviations from the normal segregation ratio in populations derived from hybrids infected with viruses were reported in maize. Barley stripe mosaic virus, Wheat streak mosaic virus and Lily ringspot virus have been shown to be responsible for the aberrant ratio phenomenon in maize [35, 36] which persists for a few years in the infection-free progeny of virus-infected plants [37]. Aberrant lines are characterized by the occurrence of unstable alleles, chromosome breaks and stable mutations. The results obtained by us during 1984-2002 are different from those obtained by the above authors and suggest that the variations observed were due to modification of crossing-over rates in virus-infected plants.

References

[1] V. Dišler: *Induced recombinogenesis in high plants*, Zinatne, Riga, 1983 (in Russian).
[2] A.A. Žučenko and A.B. Korol': *Recombination in Evolution and Breeding*, Nauca, Moscow, 1985 (in Russian).
[3] A.M. Burdun, I.V. Panarin and E.S. Zabavina: "Recombination in wheat induced by barley stripe mosaic virus", *Selekciâ i semenovodstvo*, Vol. 2, (1984), pp. 15–18 (in Russian).
[4] L. Andronic: "The tomato aspermy virus as an inducer of genetic variability in infected plants", In: *Ecologia Europaea'97, Strasbourg (France)*, 1998, pp. 1–2.
[5] A.I. Yukhimenco, S.I. Voloshchuk and V.S. Girco: "Winter wheat viruses as biological stress factors inducing genetic variation", *Bulgarian J. Plant Physiol.; Abstr. 11th Congress of the FESPP, Varna*, 1988, p. 222.
[6] L. Marii and Gh. Chiriac: "Impact of tobacco mosaic virus and potato virus X on precocity segregation at tomato" , *Res. Veg. Anim. Genet.*, Vol. 8, (2004), pp. 105–115 (in Romanian).
[7] S.A. Henderson: "Chromosome pairing, chiasmata and crossing-over", In*: Handbook of molecular cytology,* North-Holland, Amsterdam, 1969, pp. 326–357.
[8] H. Rees and P.I.Dale: "Chiasmata and variability in Lolium and Festuca population", *Chromosoma*, Vol. 17, (1974), pp. 335–351.
[9] H.K. Srivastava: "Correlation between chiasma frequency and quantitative traits in upland cotton (Gossypium hirsutum L.)", *Theor. Appl. Genet.*, Vol. 56, (1980), pp. 113–117.
[10] M. Westerman: "Chiasma frequency and variability of morphological characters in populations of two grasshopper species", *Heredity*, Vol. 51, (1983), pp. 501–506.
[11] J.G. Atherton and J. Rudich: *The tomato crop. A scientific basis for improvement*, Chapman & Hall, London, 1986.

[12] N.I. Bočârnikova and V.M. Kozlova: *Tomatoes mutant forms*, Stiinta, Chisinau, 1992 (in Russian).
[13] M.F. Clark and A.N. Adams: "Characteristics of the microplate method of enzyme-linked immunosorbent detection of plant viruses", *J. Gen. Virol*, Vol. 34, (1977), pp. 475–483.
[14] K.S. Derrick and R.H. Brlansky: "Assay for viruses and mycoplasmas using serologycally specific electron microscopy", *Phytopathology*, Vol. 66, (1976), pp. 816-820.
[15] P.Y. Paševa: *Methodology on plant cytology*, Agropromizdat, Moscow, 1988 (in Russian).
[16] H. Imai and Moriwaki: "Re-examination of chiasma terminalization and chiasma frequency in male mice", *Chromosoma*, Vol. 85, (1982), pp. 439–452.
[17] A.G. Clewer and D.H. Scarisbrick: *Practical statistics and experimental design for plant and crop science*, Wiley John & Sons, LTD, 2001.
[18] B.A. Dospehov: *Field experimental methods*, Kolos, Moscow, 1979 (in Russian).
[19] H. Hirai, M. Hirata, Y. Aoki, M. Tanaka and H.T. Imai: "Chiasma analyses of the parasite flukes, *Schistosoma* and *Paragonimus* (Trematoda), by using the chiasma distribution graph", *Genes Genet. Syst.*, Vol. 71, (1996), pp. 181–188.
[20] C.D. Darlington: *Recent Advances in Cytology*, P. Blakiston/s Soc&Co.Inc., Philadelphia, 1932.
[21] B. John: *Meiosis*, Cambridge University Press, Cambridge, 1990.
[22] J. Sherman and S. Stack: "Two-dimensional spreads of synaptonemal complex from Solonaceous plants. VI High-resolution recombination nodule map for tomato (Lycopersicon esculentum)", *Genetics*, Vol. 141, (1995), pp. 683–708.
[23] G.H. Jones: "A test for early terminalisation of chiasmata in diplotene spermatocytes of *Schistocerca gregaria*", *Chromosoma*, Vol. 63, (1977), pp. 287–294
[24] C. Tease and G.H. Jones: "Analysis of exchange in differentially stained meiotic chromosomes of *Locusta migratoria*, after BrdU-substitution and FPG staining. I. Crossover exchanges in mono chiasmate bivalents", *Chromosoma*, Vol. 69, (1978), pp. 163–178.
[25] N. Kanda and H. Kato: "Analysis of crossing over in mouse meiotic cells by BrdU labeling technique", *Chromosoma*, Vol. 78, (1980), pp. 113–121.
[26] Í¸. Rodriguiez, N. Mason, Rufas and G. De La Vega: "Effects of supernumerary heterochromatin on chiasma formation and chromosome segregation in *Dociostaurus genei* (Orthoptera)", *Heredity*, Vol. 80(3), (1998), pp. 353–360.
[27] D.L.J. Quick: *Principles and Techniques of Contemporary Taxonomy*, Blackie Academic & Professional, Glasgow, 1993.
[28] M. Sheidai, K. Mehdigholi, A. Ghahreman and F. Attar: "Cytogenetic study of the genus *Cousinia* (Asteraceae, section *Serratuloideae*) in Iran", *Genet. Mol. Biol.*, Vol. 29 , (2006).
[29] R.N. Jones and H. Rees: *B-Chromosomes*, Academic Press, New York, 1982.

[30] A.B. Korol, I.A. Preygel and S.I. Preygel: *Recombination variability and evolution. Algorithms of estimation and population-genetic models*, Chapman & Hall, London, 1994.

[31] O. Bell and A. Burt: "B-chromosomes: germ-line parasites with induced changes in host recombination", *Parasitology*, Vol. 100, (1990), pp. 519–526.

[32] T.O. Dinner: "Physiology of virus-infected plants", *Annu. Rev. Phytopathol,* Vol. 1, (1963), pp. 197–218.

[33] I. Stokes, R. Mock and A. Gillaspie: "Influence of Sugarcane Mosaic Virus on Segregation for two Seedling Characteristics in Sorghum", *Crop Sci.*, Vol. 28, (1988), pp. 443–446.

[34] A.A. Žučenko: *Adaptive system of plant selection (ecologic-genetic fundamentals*), Publishing House Agrorus Ltd., Moscow, 2001, (in Russian).

[35] G. Sprague and H.H. McKinney: "Further evidence on the genetic behavior of AR in maise", *Genetics*, Vol. 67, (1971), pp. 533–542.

[36] R.G. Samson, M.K. Brakke and W.A. Compton: "Evidence for gene inactivation in the virus-induced aberrant ratio phenomenon in maize", *Genetics*, Vol. 92, (1979), pp. 1231–1239.

[37] M.K. Brakke and R.G. Samson: "Effect of temperature on susceptibility of normal and aberrant ratio corn stocks to barley stripe mosaic and wheat streak mosaic viruses", *Phytopathology*, Vol. 71, (1981), pp. 823–824.