MEIOTIC ANALYSIS IN THE INDUCED MUTANTS OF ROSA HYBRIDA CV. FOLKLORE

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ABSTRACT

Microsporogenesis was studied in rose. cv. Folklore and its twelve gamma-ray induced mutants. Different types of meiotic irregularities, viz., multivalents, univalents, fragments, laggards and anaphase bridges were commonly observed both in cv Folklore as well as in its induced mutants. Maximum number of bivalents per PMC were recorded in Climbing Orange-White which was significantly higher than the mutants Solid Orange and Striped Pinkish-Red, as compared to control. Higher meiotic irregularities in the induced mutants may be due to complicated structural changes and alteration in karyotype which caused variation in the morphology of the mutants.

Key words: Rose, meiosis, chromosomal aberrations, induced mutants.

Garden roses are complex hybrids with the basic chromosome number X = 7. Cytological investigations in Rosa were initiated in 1920 identifying polyploid series in this genus [1, 2]. The phenomenon of meiosis is controlled by several major genes [3, 4]. Most of the mutations that have been reported so far affect chromosome pairing influencing chiasma frequency and formation of multivalents, bivalents and univalents [5–9]. Rose cv. Folklore is a commercially exploited hybrid (2n = 28). The present investigation deals with different types of chromosomal aberrations observed at diakinesis and mutaphase I of meiosis in this variety and its induced mutants.

MATERIALS AND METHODS

The material comprised cv. Folklore and its 12 induced mutants, namely, Apricot, Yellow, Pink, Orange, Solid Orange, Pink Tipped Orange, Striped Red (stripes on the reverse of petals), Striped Orange-Red, Striped Pinkish-Red, Dwarf Striped Pink, Dwarf Bright-Yellow and Climbing Orange-White [10–12]. For cytological studies, young flower buds of appropriate size were collected and pre-fixed in Carnoy's fluid (6:3:1; absolute ethanol:

August, 1993]

Meiotic Analysis in Roses

chloroform:acetic acid) for 1 h, then kept in acetic alcohol (1:3) having ferric chloride for 2 h, and then changed to acetic alcohol (1:3) (having no ferric chloride) for 24 h. The fixed material was stored in refrigerator in 70% ethyl alcohol. The anther's were squashed in 1% propiono-carmine after hydrolysis in a mixture of 12 drops of 2% propiono-carmine + 3 drops of 1 NHCl for 20 min at 60° C. Twenty five pollen mother cells (PMCs) at diakinesis/metaphase I were examined at random and micro-photographs taken from the temporary slides. The data on mean number of bivalents, multivalents and chiasmata per cell were subjected to statistical analysis for determining the significance in differences (SE and CD).

RESULTS AND DISCUSSION

Different types of chromosomal configurations were observed in the induced mutants as well as control (Fig. 1: 1–9).

BIVALENTS

Significant differences between induced mutants and the control were observed pertaining to ring, rod and precociously separating bivalents, and also between their chiasma frequencies (Table 1). The highest number of ring bivalents per PMC (2.96) was

Mutant	Bivalent associations, No./Cell								
		ring		r	precociously				
	4 xta	3 xta	2 xta	2 xta	1 xta	separating			
						1 xta			
Apricot	—	0.28 + 0.24	1.92 + 0.44	0.16 <u>+</u> 0.09	8.56 + 0.70	0.56 <u>+</u> 0.19			
Yellow		0.04 <u>+</u> 0.04	0.68 <u>+</u> 0.19	0.16 <u>+</u> 0.08	10.56 <u>+</u> 0.50	0.20 <u>+</u> 0.10			
Pink			1.16 <u>+</u> 0.23	0.12 <u>+</u> 0.07	11.28 + 0.35	0.08 <u>+</u> 0.06			
Orange		_	0.84 <u>+</u> 0.15	_	11.80 <u>+</u> 0.40	_			
Solid Orange	0.08 <u>+</u> 0.06	—	0.92 <u>+</u> 0.19	0.08 <u>+</u> 0.06	9.24 <u>+</u> 0.55	0.08 <u>+</u> 0.20			
Pink Tipped Orange			1.48 + 0.23	******	11.22 <u>+</u> 0.39	0.12 <u>+</u> 0.04			
Striped Red	0.20 <u>+</u> 0.10		0.92 + 0.23	_	10.40 + 0.44	0.24 + 0.13			
Striped Orange-Red	0.04 <u>+</u> 0.04	0.12 <u>+</u> 0.07	1.04 <u>+</u> 0.20	0.08 + 0.06	10.00 <u>+</u> 0.47	0.48 <u>+</u> 0.21			
Striped Pinkish-Red		-	1.68 + 0.32	0.16 <u>+</u> 0.08	9.24 <u>+</u> 0.46	0.16 <u>+</u> 0.09			
Dwarf Striped Pink	0.08 <u>+</u> 0.06		1.28 + 0.18	_	11.44 <u>+</u> 0.33	0.16 <u>+</u> 0.09			
Dwarf Bright-Yellow	0.24 <u>+</u> 0.13		2.56 + 0.31	_	9.32 <u>+</u> 0.45	0.24 + 0.15			
Climbing Orange-White	0.08 <u>+</u> 0.16	_	2.88 + 0.28		10.24 + 0.38				
Control (cv. Folklore)	0.12 <u>+</u> 0.67	0.04 <u>+</u> 0.04	1.80 + 0.25	0.24 <u>+</u> 0.12	9.80 <u>+</u> 0.30	0.60 + 0.23			

Table 1 Chromosomal pairing of bivalents and chiasma frequency in rose cv. Folklore and its induced mutants



Meiotic patterns in the mutants of rose. (1) Normal pairing (14 II) in control. (2) Ring bivalent and quadrivalent chromosome configurations in 'Dwarf Bright-Yellow' mutant. (3) Rod bivalent chromosome configuration in Dwarf Bright-Yellow mutant. (4) Precociously separating bivalents in Solid Orange mutant. (5) Precociously separating bivalent in Dwarf Striped Pink mutant. (6) PMC showing complete asynapsis (28 IS) in Apricot mutant. (7) Chromosome fragment in Dwarf Striped Pink mutant. (8) Anaphase bridges in Striped Pinkish-Red mutant. (9) Laggard in Apricot mutant. Magnification x3500.

August, 1993]

Meiotic Analysis in Roses

recorded in the mutant Climbing Orange-White and lowest in Yellow as compared to control (1.96); complete pairing of chromosomes in the former was observed as compared with the control. In case of rod bivalents, maximum number per PMC (11.8) was recorded in the mutant Orange as against 10.0 in the control. Climbing Orange-White mutant showed maximum bivalent pairing (13.2/PMC) and the minimum number of bivalents was recorded in Solid Orange (Table 2). The highest number of chiasmata (18.5/PMC) was recorded in the mutant Dwarf Bright-Yellow and minimum (15.7/PMC) was in Orange. It is known that some major gene(s) control chiasma frequency [13] which could be due to homogeneity of the mutants, temperature and environmental conditions [14–16].

MULTIVALENTS

Solid Orange mutant exhibited maximum (1.3) and Climbing Orange-White minimum (0.4) number of multivalents per cell (Table 2). There was no indication of trivalent formation in this climbing mutant (Table 3). The multivalent frequency increases at the expense of bivalents [7, 17].

Mutant	Univalents	Fragments	Bivalents	Multi- valents	Chromosomes associated as multi- valents	Chiasmata per cell
Apricot	2.64 <u>+</u> 1.42		11.5 <u>+</u> 0.53	0.64 + 0.03	5.50 <u>+</u> 0.84	16.1 <u>+</u> 0.68
Yellow	0.36 <u>+</u> 0.14	_	11.6 <u>+</u> 0.45	1.04 <u>+</u> 0.05	8.00 + 1.07*	16.2 <u>+</u> 0.36
Pink	0.12 <u>+</u> 0.09	_	12.6 <u>+</u> 0.35	0.50 <u>+</u> 0.05	5.44 <u>+</u> 0.65	16.4 <u>+</u> 0.36
Orange	0.20 <u>+</u> 0.08		12.6 <u>+</u> 0.36	0.64 <u>+</u> 0.03	5.56 <u>+</u> 1.00	15.7 <u>+</u> 0.32
Solid Orange	0.92 <u>+</u> 0.08	_	11.1 <u>+</u> 0.59 [*]	1.28 + 0.06	8.56 + 2.05	16.6 <u>+</u> 0.33
Pink Tipped Orange	0.40 <u>+</u> 0.17	0.04 <u>+</u> 0.04	12.7 <u>+</u> 0.41	0.60 <u>+</u> 0.04	6.00 <u>+</u> 1.24	16.3 <u>+</u> 0.35
Striped Red	0.20 <u>+</u> 0.12		11.8 <u>+</u> 0.41	1.08 <u>+</u> 0.08	6.56 <u>+</u> 1.09	17.0 <u>+</u> 0.43
Striped Orange-Red	1.36 <u>+</u> 0.13	0.08 <u>+</u> 0.06	11.8 <u>+</u> 0.43	1.00 <u>+</u> 0.05	7.22 <u>+</u> 1.01	17.1 <u>+</u> 0.33
Striped Pinkish-Red	1.32 <u>+</u> 0.26	—	11.2 <u>+</u> 0.47 [*]	1.12 <u>+</u> 0.05	5.89 <u>+</u> 1.03	17.0 <u>+</u> 0.47
Dwarf Striped Pink	0.36 <u>+</u> 0.17	0.08 + 0.00	13.0 <u>+</u> 0.31	0.44 <u>+</u> 0.03	4.78 <u>+</u> 0.52	16.0 <u>+</u> 0.27
Dwarf Bright-Yellow	0.16 <u>+</u> 0.09	0.04 <u>+</u> 0.04	12.4 <u>+</u> 0.36	0.76 <u>+</u> 0.05	5.66 <u>+</u> 0.97	18.5 <u>+</u> 0.53 [*]
Climbing Orange-White		_	13.2 <u>+</u> 0.23 [*]	0.40 + 0.04	4.44 <u>+</u> 0.44	17.9 <u>+</u> 0.41 [*]
Control (cv. Folklore)	0.48 <u>+</u> 0.17	_	12.6 <u>+</u> 0.32	0.60 <u>+</u> 0.04	4.78 + 0.62	17.2 <u>+</u> 0.36
CD		-	1.18		2.96	1.2

 Table 2. Mean number of different chromosomal associations and chiasmata per cell in Rosa hybrida

 cv. Folklore and its induced mutants

^{*}Significant at 1% level.

UNIVALENTS AND FRAGMENTS

Completely desynaptic type metaphase I (showing 28 MI instead of 14 MII) was observed in a few PMCs of the mutant Apricot (Fig. 1: 6). This mutant produced highest number of univalents and laggards in contrast to Climbing Orange-White (no univalents). Fragments were also observed in some of the mutants (Fig. 1: 7) except Climbing Orange-White and control (Table 3). The univalents are the primary source of laggards [9]. The increase in the number of univalents could be due to structural changes caused by irradiation or a gene mutation controlling pairing [6]. The behaviour of fragments varies in different materials, these may either pair with the normal chromosomes giving rise to multivalents or may be observed as free small dots as compared to the major chromosomes [8]. An addition or deletion of a chromosome may affect the expression of some morphological characters, i.e. prickle-density, pattern of branching, leaf characters and flower morphology.

Mutant	Octa- valents	Hexa- valents	Penta- valents	Quadri- valents	Tri- valents	Early sepa- rating bivalents	MI uni- valents	Frag- ments	Lagg- ards	Ana- phase bridges
Apricot	_	4		32	24	32	48	_	13.05	5.4
Yellow	_	20		48	16	16	24		5.4	
Pink	4		4	40		8	8		8.3	2.8
Orange	4		—	32	20		20		9.7	2.9
Solid Orange			8	56	16	44	40	_	3.3	1.5
Pink Tipped Orange		—	_	32	8	8	20	4	6.1	3.0
Striped Red				68	4	12	12		2.9	2.9
Striped Orange-Red	4	8		48	20	20	28	8	5.6	
Striped Pinkish-Red		8	4	40	44	12	64	—	9.1	3.0
Dwarf Striped Pink	_	_	_	36	4	12	16	8	3.6	_
Dwarf Bright-Yellow	4			48	8	12	12	4	6.9	3.5
Climbing Orange-White	_	_	_	36			_	_	9.5	4.8
Control (cv. Folklore)		_	_	40	4	28	24	_	4.2	4.2

Table 3.	Chromosomal aberrations	per 100 cells in rose cv	. Folklore and	its induced	mutants
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296

August, 1993]

Meiotic Analysis in Roses

CHROMOSOMAL ABERRATIONS

Different types of meiotic abnormalities were observed in different frequencies in both induced mutants as well as in control (Table 3). Octa., hexa- and pentavalents were observed in a few PMCs of some of the mutants. Multivalents, viz., quadri- and trivalents were commonly observed in all the mutants and the control (cv. Folklore) which is an inter-specific hybrid. Multivalent formation in an interspecific hybrid indicates the existence of interchromosomal translocation or duplication [18]. The presence of multivalents may also indicate that induced complicated structural changes took place during the natural hybridization [6] or mutation induction in the rose cv. Folklore. The higher percentage of quadri- and trivalents along with univalents in rose cv. Folklore shows that it is basically a segmental alletetraploid. The climbing habit could be correlated with complete pairing of chromosomes [19, 20] as the Climbing Orange-White mutant recorded complete pairing of chromosomes without any trivalents, univalents and fragments at meiosis I.

Other chromosomal aberration, viz. fragments, laggards and anaphase bridges were also recorded in some of the mutants. Such chromosomal abnormalities may be the direct consequences of radiation treatment.

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Surendra Kumar et al.

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