MICROSPOROGENESIS IN GINGER (*ZINGIBER OFFICINALE* ROSC.)

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ABSTRACT

Intraspecific variability for meiotic behaviour has been investigated in 25 cultivars of *Z. officinale*. The presence of quadrivalents in most of the cultivars of *Z. officinale* and hexavalents in few, is presumed to be due to translocation involving four to six chromosomes.

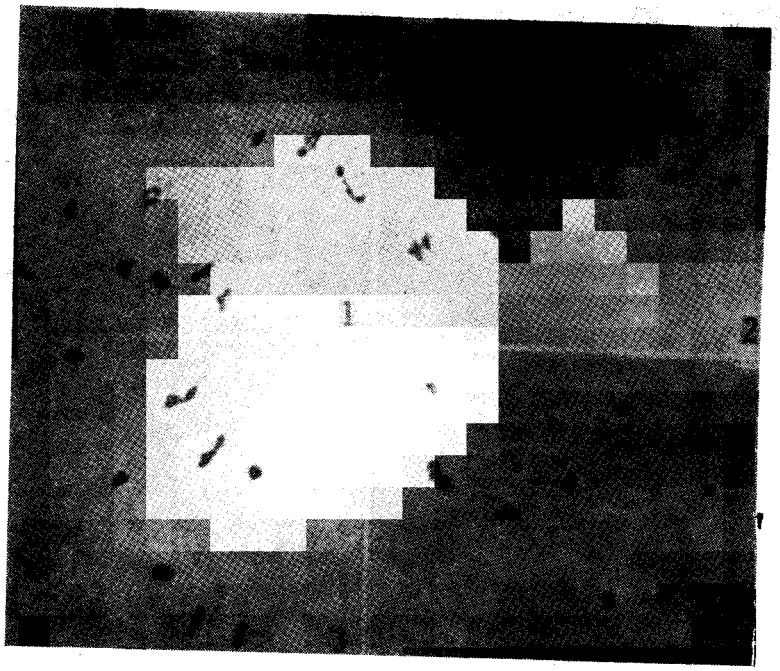
A significant positive linear regression between pollen sterility, and chromosome abberration at anaphase-II and aberrant quartets has been established. It is inferred that the structural chromosome aberrations have a significant influence in lowering the fertility in cultivars of *Z. officinale*.

Based on earlier reports and present investigation, a basic chromosome number of x = 11 has been inferred for the genus *Zingiber*.

INTRODUCTION

The first detailed cytological study in the family Zingiberaceae was by Raghavan and Venkatasubban (1943), who reported the chromosome number and morphology of three species *Zingiber*, (*Z. officinale* Rosc., *Z. cassumunar* Roxb. and *Z. zerumbet* Sm.) along with few species of *Costus*, *Kaemferia*, *Alpinia*, *Circuma* and *Hedychium*. Prior to that, chromosome number for *Z. officinale* was reported by Morinaga *et al.* (1929), Takahashi (1930) and Sugiura (1936) and for *Z. mioga* Rosc. by Morinaga *et al.* (1929). Chakravorti (1948) reported a chromosome number of 2n=22 for *Z. officinale* and he concluded that in view of the normal pairing of 11 bivalents in species like *Z. cassumunar* and *Z. zerumbet*, *Z. moga* with 2n = 55 chromosomes was to be considered as a pentaploid. Ramachandran (1969) found regular meiosis with good pollen and seed set in Z. *roseum* Rosc., *Z. wightianum* Thw. and *Z. zerumbet* Sm.

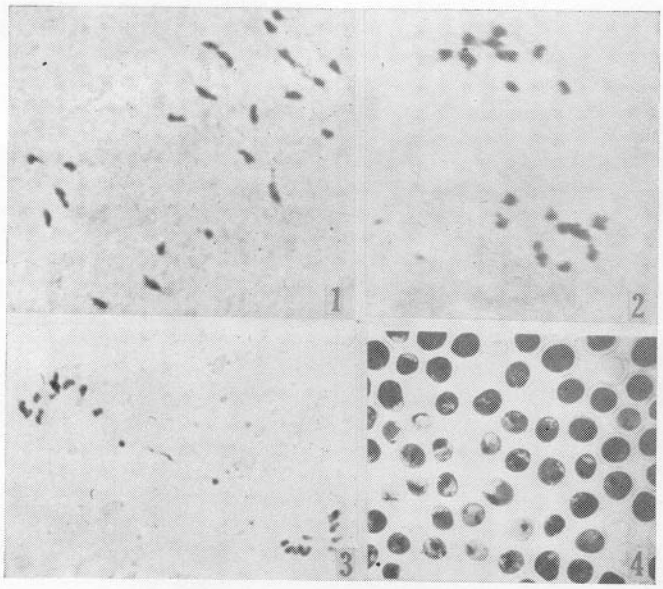
PLATE 1



Microsporogenesis in *Z. officinale*

Fig. 1. Diakinesis $10^{ii}+2^{i}$
Fig. 2. Metaphase I, 11^{ii}
Fig. 3. Metaphase I, $3^{iv}+5^{ii}$
Fig. 4. Metaphase I, $1^{vi}+5^{ii}$

PLATE 2



Microsporogenesis in *Z. officinale* (Contd.)

Fig. 1. Anaphase I
Fig. 2. Anaphase I } normal separation
Fig. 3. Anaphase I, showing leggards
Fig. 4. Polen grains.

Microsporogenesis in 25 cultivars of *Z. officinale* and two wild species (*Z. zerumbet* and *Z. cassumunar*) is reported in this paper.

Materials and Methods

For the study of microsporogenesis, the flower buds were collected between 11.00 and 12.00 h and fixed in Carnoy's fluid. The materials were retained for 24 hours in the fixative and later washed and stored in 70% alcohol. The anthers were squeezed in 1% acetocarmine. The meiotic cells were analysed from the temporary preparations and were made permanent following the method of Swaminathan *et al.* (1954). Pollen fertility was studied in 1% acetocaramine glycerine (1 : 1).

Simple linear regression of pollen sterility, anaphase-I abnormalities, second division abnormalities and abnormal tetrads were worked out separately based on percentage values and transformed (angular) values.

Results and Discussion

All the 25 cultivars of *Z. officinale*, and two related species *Z. Zerumbet*, *Z. cassumunar* had chromosome number of n=11 (Plate 1; Fig. 2). Considerable variability for chromosome association was observed among the cultivars of *Z. officinale*. The maximum association of one hexavalent was observed in cultivars 'Bangkok', 'Taiwan', 'Sierra Leone', 'Mananthody', 'Ernad Chernad', 'Valluvanad', 'Jorhat', 'Thingpuri', 'Maran', and two wild species (Plate 1; Fig. 4). Quadrivalents ranging from one to three were observed in almost all the cultivars (Plate 1; Fig. 3) except in 'Tafingiva' and 'Karakkal' and the latter had only bivalents both at diakinesis as well as metaphase-I. The highest frequency of 11 bivalents at diakinesis was noticed in all the cultivars except 'China', 'Sierra Leone', and 'Mananthody'. Considerable variation was also noticed in the number of univalents among the species and cultivars investigated (Plate 1; Fig. 1). Range and mean of chromosome associations observed at diakinesis and metaphase-I are given in Table 1.

Meiotic abnormalities to varying extent were observed in the first division as well as second division and the details are given in

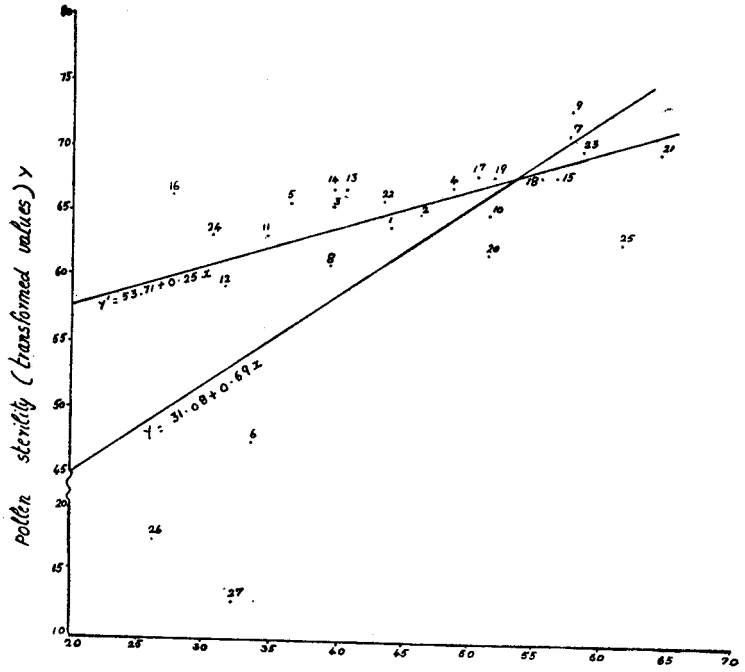
Table 1. Chromosome association at diakenesis and metaphase-I in cultivars of *Z. officinale* and two species of *Zingiber*
(Figures in parenthesis denote values after angular transformation)

Sl. No.	Species/ cultivars	DIAKINESIS							METAPHASE						
		No. of PMCs observed	VI Range (mean)	V Range (mean)	IV Range (mean)	III Range (mean)	II Range (mean)	I Range (mean)	No. of PMCs observed	VI Range (mean)	V Range (mean)	IV Range (mean)	III Range (mean)	II Range (mean)	I Range (mean)
I. Z. officinale															
1.	China ..	41	—	—	0-1 (0.54)	—	7-10 (9.51)	0-2 (0.82)	80	—	—	0-2 (0.34)	—	8-11 (9.91)	0-4 (0.82)
2.	Bangkok ..	78	—	—	0-1 (0.32)	—	9-11 (10.10)	0-4 (0.52)	68	0-1 (0.01)	—	0-1 (0.19)	—	7-11 (10.25)	0-4 (0.72)
3.	Taiwan ..	26	0-1 (0.04)	—	0-2 (0.23)	—	4-11 (10.38)	0-2 (0.08)	76	0-2 (0.04)	—	0-2 (0.12)	—	3-11 (10.45)	0-2 (0.38)
4.	Sierra Leone	45	—	—	0-2 (0.78)	—	7-11 (9.07)	0-2 (0.74)	49	0-1 (0.02)	0-1 (0.02)	0-2 (0.55)	0-1 (0.04)	6-11 (9.30)	0-4 (0.86)
5.	Tafingiva ..	36	—	—	—	—	10-11 (10.83)	0-2 (0.34)	63	0-1 (0.02)	—	0-2 (0.06)	0-1 (0.02)	7-11 (10.00)	0-6 (1.54)
6.	Jamaica ..	46	0-1 (0.02)	—	0-2 (0.87)	—	3-11 (9.18)	0-2 (0.04)	55	0-1 (0.04)	—	0-2 (0.27)	—	3-11 (10.18)	0-2 (0.32)
7.	Rio de Janeiro	35	0-1 (0.06)	—	0-3 (0.57)	—	4-11 (9.68)	—	53	—	—	0-3 (0.28)	0-1 (0.04)	5-11 (9.87)	0-6 (1.02)
8.	Wynaad Local	20	—	—	0-1 (0.10)	—	9-11 (10.80)	—	47	—	—	0-1 (0.06)	—	9-11 (10.24)	0-4 (1.28)
9.	Wynaad Kunna-mangalam	44	—	—	0-2 (0.50)	—	7-11 (10.00)	—	46	—	—	0-2 (0.24)	—	7-11 (10.15)	0-4 (0.74)
10.	Mananthody	46	0-1 (0.06)	—	0-2 (0.50)	0-2 (0.13)	7-11 (9.56)	0-2 (0.13)	53	—	—	0-1 (0.24)	0-2 (0.07)	8-11 (10.34)	0-2 (0.15)
11.	Kuruppampadi	47	—	—	0-3 (0.34)	—	5-11 (10.17)	0-2 (0.30)	75	—	—	0-1 (0.09)	0-1 (0.01)	7-11 (10.12)	0-4 (1.37)

12.	Ernad Manjeri	59	—	—	0-1 (0.41)	—	8-11 (9.98)	0-2 (0.40)	69	—	—	0-1 (0.14)	—	7-11 (10.07)	0-4 (13.30)
13.	Ernad Chernad	85	0-2 (0.02)	—	0-3 (0.58)	—	3-11 (9.78)	—	42	—	—	0-3 (0.33)	—	5-11 (10.08)	0-4 (0.52)
14.	Valluvanad	63	0-1 (0.02)	—	0-2 (0.14)	—	4-11 (10.46)	0-6 (0.40)	117	0-1 (0.02)	—	0-3 (0.11)	—	5-11 (10.42)	0-6 (0.60)
15.	Thodupuzha	50	—	—	0-1 (0.01)	—	9-11 (10.98)	—	112	—	—	0-1 (0.05)	—	7-11 (10.30)	0-4 (1.20)
16.	Vengara ..	40	—	—	0-1 (0.20)	—	9-11 (10.55)	0-2 (0.10)	35	—	—	0-1 (0.23)	—	9-11 (10.48)	0-2 (0.12)
17.	Karaikkal ..	63	—	—	—	—	10-11 (10.80)	0-2 (10.10)	79	—	—	—	—	9-11 (10.16)	0-4 (1.68)
18.	Uttar Pradesh	30	—	—	0-1 (0.57)	—	8-11 (9.43)	0-2 (0.86)	43	—	—	0-2 (0.16)	—	7-11 (9.65)	0-4 (2.06)
19.	Bajpai ..	55	—	—	0-3 (0.33)	0-1 (0.02)	5-11 (0.25)	0-2 (0.12)	73	—	—	0-1 (0.15)	0-1 (0.04)	8-11 (10.14)	0-4 (1.00)
20.	Assam ..	28	—	—	0-1 (0.25)	—	9-11 (10.25)	0-2 (0.50)	73	—	—	0-3 (0.16)	0-1 (0.04)	4-11 (10.00)	0-6 (1.24)
21.	Jorhat ..	45	0-1 (0.04)	—	0-2 (0.62)	0-1 (0.02)	1-11 (9.60)	0-1 (0.02)	114	0-1 (0.06)	—	0-3 (0.22)	0-1 (0.06)	5-11 (9.54)	0-4 (0.70)
22.	Thingpuri ..	60	0-1 (0.02)	—	0-2 (0.30)	—	8-11 (10.23)	0-2 (0.22)	63	—	—	0-2 (0.32)	—	7-11 (9.90)	0-6 (0.92)
23.	Jugijan ..	32	—	—	0-1 (0.32)	—	8-11 (10.00)	0-2 (0.72)	89	—	—	0-3 (0.37)	—	5-11 (10.00)	0-4 (0.52)
24.	Burdwan ..	51	—	—	0-1 (0.21)	—	8-11 (10.32)	0-4 (0.52)	33	—	—	0-1 (0.39)	—	8-11 (9.91)	0-2 (0.62)
25.	Maran ..	102	0-1 (0.02)	—	0-2 (0.08)	—	4-11 (10.69)	0-2 (0.18)	70	0-1 (0.01)	—	0-2 (0.04)	—	4-11 (10.75)	0-2 (0.28)
II.	*Z. zerumbet* ..	47	0-1 (0.11)	—	0-1 (0.46)	—	6-11 (9.75)	—	110	0-1 (0.03)	—	0-2 (0.22)	—	7-11 (10.33)	0-4 (0.28)
III.	*Z. cassumunar*	55	0-1 (0.05)	—	0-1 (0.29)	—	6-11 (10.27)	—	64	0-1 (0.02)	—	0-1 (0.20)	—	8-11 (10.39)	0-4 (0.30)

Table 2 and Plate 2. Simple linear regression of pollen sterility on anaphase-I abnormalities, second division abnormalities and aberrant tetrads were worked out, based on percentage values and transformed (angular) values and the results of analysis are given in Table 3. Since the fitness was found to be more appropriate with

PLATE .3



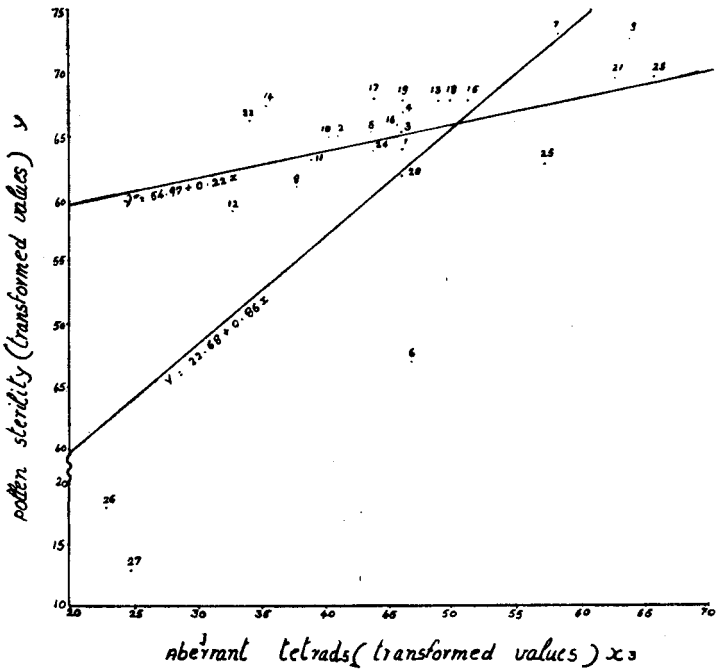
Scatter diagram showing the relation between second division meiotic abnormalities (%) and pollen sterility (%) in 25 cultivars of *Z. officinale* and two *Zingiber* species.

transformed values, the scatter diagrams were drawn using transformed values only. It was observed from the scatter diagrams that *Z. zerumbet* and *Z. cassumunar* were far removed from the estimated linear regression equation. Hence revised linear regression parameters were worked out excluding these two species and the results are presented in Table 4. Significant positive linear regression was found between pollen sterility and second division

aberrations and aberrant tetrads. Scatter diagrams for explaining the relationship between these factors are presented in Plates 3 and 4.

A somatic chromosome number of 2n = 22 has been reported in literature for all the species of *Zingiber* except for *Z. mioga* with a basic number of $x = 11$ for the genus (Sato, 1960; Ramachandran, 1969; Mahanty, 1970). The presence of multivalents in the diploid species of Zingiber in the present investigation shows

PLATE - 4



Scatter diagram showing the relation between aberrant tetrads (%) and pollen sterility (%) in 25 cultivars of *Z. officinale.* and two *Zingiber* species.

that probably they are structural heterozygotes involving segmental interchanges. The presence of quadrivalents in most of the cultivars of *Z. officinale* and hexavalents in few shows that at least four to six chromosomes are involved in the trasnslocations.

The alterations in the chromosome associations of different cultivars of *Z. officinale* which consisted of univalents, trivalents,

Table 2. Relation between anaphase-I abnormalities, second division abnormalities, abnormal tetrads and pollen sterility in cultivars of *Z. officinale* and two wild species of *Zingiber*

(Figures in parenthesis denote values after angular transformation)

Sl. No.	Species/cultivars	PMCs with anaphase-I abnormalities (%)	PMCs with second division abnormalities (%)	Abnormal (%)	Pollen sterility (%)
I.	*Z. officinale*				
1.	China ..	31.0 (33.83)	45.8 (42.59)	52.9 (46.66)	81.0 (64.16)
2.	Bangkok ..	33.3 (35.24)	51.7 (45.97)	42.9 (40.92)	82.0 (64.90)
3.	Taiwan ..	8.1 (16.54)	40.0 (39.23)	51.9 (46.09)	82.8 (65.50)
4.	Sierra Leone ..	47.2 (43.39)	55.1 (47.93)	52.2 (46.26)	85.0 (67.21)
5.	Tafingiva ..	11.0 (19.37)	34.4 (35.91)	48.8 (44.31)	82.9 (65.57)
6.	Jamaica ..	11.6 (19.91)	30.2 (33.34)	54.2 (47.41)	54.4 (47.52)
7.	Rio de Janeiro ..	17.6 (24.8)	71.2 (57.54)	70.6 (57.17)	90.2 (71.76)
8.	Wynaad Local ..	5.5 (13.56)	39.4 (38.88)	37.9 (38.00)	76.7 (61.14)
9.	Wynaad Kunnamangalam ..	15.4 (23.11)	70.7 (57.23)	80.5 (63.79)	91.4 (72.95)
10.	Mananthody ..	21.1 (27.35)	60.2 (50.89)	42.2 (40.51)	82.5 (65.27)

1	Kuruppampadi	..	24.5 (29.67)	30.8 (33.71)	39.7 (39.06)	79.6 (63.15)
12.	E. ad Manjeri	..	18.0 (25.10)	26.9 (31.24)	30.3 (33.40)	74.2 (59.47)
13.	Ernad Chernad	..	21.4 (27.56)	41.5 (40.11)	56.7 (48.85)	84.4 (66.74)
14.	Valluvanad	..	20.0 (26.57)	39.4 (38.88)	33.8 (35.55)	85.6 (67.70)
15.	Thodupuzha	..	9.8 (18.24)	69.2 (56.29)	61.2 (51.47)	86.4 (68.36)
16.	Vengara	..	3.2 (10.30)	20.6 (26.99)	51.9 (46.09)	84.0 (66.42)
17.	Karakkal	..	20.7 (27.06)	58.3 (49.78)	48.7 (44.26)	85.7 (67.78)
18.	Uttar Pradesh	..	32.9 (35.00)	67.0 (54.94)	58.8 (50.07)	86.1 (68.11)
19.	Bajpai	..	34.2 (35.79)	61.4 (51.59)	5.5 (45.86)	85.5 (67.62)
20.	Assam	..	38.4 (38.29)	61.2 (51.47)	51.6 (45.92)	78.5 (62.38)
21.	Jorhat	..	18.7 (25.62)	81.1 (64.23)	79.4 (63.01)	88.7 (70.36)
22.	Thingpuri	..	21.4 (27.56)	47.2 (43.39)	24.3 (29.53)	84.0 (66.42)
23.	Jugijan	..	19.7 (26.35)	72.4 (58.31)	83.2 (65.80)	88.8 (70.45)
24.	Burdwan	..	23.1 (28.73)	24.6 (29.73)	47.9 (43.80)	79.6 (63.15)
25.	Maran	..	19.4 (26.13)	76.6 (61.07)	71.2 (57.53)	79.3 (62.94)
II.	*Z. zerumbet*	..	28.4 (32.20)	19.3 (26.06)	15.2 (22.95)	9.0 (17.46)
III.	*Z. cassumunar*	..	23.0 (28.66)	28.1 (32.01)	18.3 (25.33)	4.7 (12.52)

Table 3. Summary table of linear regression analysis of pollen sterility (y) on anaphase-I abnormalities (x_1), second division abnormalities (x_2) and aberrant tetrads (x_3)

For data based on 25 cultivars of Z. officinale and two species

		For percentage data			For transformed data		
		Pollen sterility *vs.* anaphase-I abnormalities	Pollen sterility *vs.* second division abnormalities	Pollen sterility *vs.* aberrant tetrads	Pollen sterility *vs.* anaphase-I abnormalities	Pollen sterility *vs.* second-division abnormalities	Pollen sterility *vs.* aberrant tetrads
1. Correlation coefficient	r	0.1707 NS	0.2293 NS	0.7293**	—0.0841 NS	0.5391**	0.6462**
2. Regression co-efficient	b	0.5094 NS	0.3763 NS	1.2747**	—0.1600 NS	0.6904**	0.8648**
3. Standard Error of	b	0.5879	0.3192	0.2388	0.3786	0.2157	0.2042
4. Regression equation : $y=a+bx$		$y=62.79+0.51x$	$y=55.25+0.38x$	$y=9.60+1.27x$	$y=57.44-0.16x$	$y=31.08+0.69x$	$y=22.68+0.86x$

** Significant at 1% level. * Significant at 5% level. NS—Not significant.

Table 4. Summary table of linear regression analysis of pollen sterility (y) on anaphase-I abnormalities (x_1), second division abnormalities (x_2) and aberrant tetrads (x_3)

For data based on 25 cultivars of Z. officinale

		For percentage data			For transformed data		
		Pollen sterility *vs.* anaphase-I abnormalities	Pollen sterility *vs.* second division abnormalities	Pollen sterility *vs.* aberrant tetrads	Pollen sterility *vs.* anaphase-I abnormalities	Pollen sterility *vs.* second division abnormalities	Pollen sterility *vs.* aberrant tetrads
1. Correlation coefficient	r	0.2331 NS	—0.0233 NS	0.5907**	0.1239 NS	0.5559**	0.4164*
2. Regression coefficient	b	0.5836 NS	—0.0323 NS	0.9609**	0.0789 NS	0.2579*	0.2243**
3. Standard Error of	b	0.5072	0.2888	0.2736	0.1318	0.0804	0.1021
4. Regression equation : $y=a'+b'x$		$y=66.56+0.58x$	$y'=80.71-0.03x$	$y'=28.15+0.96x$	$y'=63.38+0.08x$	$y'=53.71+0.26x$	$y'=54.97+0.22x$

** Significant at 1% level, * Significant at 5% level, NS—Not significant.

quadrivalents, and hexavalents, besides bivalents were possibly the outcome of irregular pairing of chromosomes due to translocations as reported in Capsicum (Katiyar, 1978). The trivalents associated with univalents were likely to be the result of translocation involving three of the two pairs of chromosomes or due to early terminalization of one of the chromosomes of a quadrivalent. Spontaneous chromosomal interchanges in *Citrus assamensis*, a diploid species has been reported by Naithani and Raghuvanshi (1958 a, b) and Raghuvanshi (1962 a, b), wherein formation of trivalents, quadrivalents, and hexavalents at metaphase-I of microsporogenesis has been observed. A ring or chain of four chromosomes was reported in diploid *Chrysanthemum carinatum* (Rana and Jan 1965) and in diploid garden *Canna* (Khushoo and Mukherjee 1966). Sisodia (1970) reported multivalent associations at diakinesis and at metaphase-I in the diploid monotypic genus *Thelepogon elegans* and assumed interlocking of bivalents at meiosis to explain these associations. Since there was no evidence for spontaneous association between heterochromatic region or spontaneous chromosome breakages, the formation of multivalents in *Z. officinale* and other species may be explained based on the reciprocal translocation and this assumption has further been supported by high pollen sterility observed in the present study. The possibilities of multivalent formation due to accidental interlocking of bivalents, and sticky nature of the chromosomes also cannot be ruled out.

Structural chromosome aberrations occurred in all stages of microsporogenesis in cultivars of *Z. officinale*. Analysis of data showed a significant positive linear regression between pollen sterility and chromosome aberrations at anaphase-II as well as pollen sterility and aberrant quartets. However, it was found that two related species, *Z. zerumbet* and *Z. cassumunar* were far-removed from the estimated regression equation. This was expected since these two species have high pollen fertility (95.3% and 91.0%), compared to *Z. officinale* (pollen fertility 8.6% to 45.6%). The results of these modified regression analysis excluding two wild species revealed that a high positive correlation exists between pollen sterility and meiotic abnormalities (Plate 2 and 3). Similar relationship has been demonstrated in *Bromus inermis* (Jalal and Nelson, 1965; Lafleur and Jalal, 1972) and in different

species *Agropyron* (Johnson and Jalal, 1977). Second division abnormalities and abnormal quarters appear to be good indicators for pollen sterility in different cultivars of *Z. officinale* and it can be concluded that structural chromosomal aberrations have significant influence in reducing fertility in them.

Chromosome numbers ranging from $2n = 18$ to 104 with multiples of 9, 11, 12 and 13 have been reported so far for 74 species belonging to eleven genera of Zingiberaceae including nine species of *Zingiber* (Raghavan and Venkatasubban, 1943, Sato, 1948; Sharma and Bhattacharya, 1959; Ramachandran, 1969; Mahanty, 1970). Among the nine species of *Zingiber* for which chromosome numbers have been reported, *Z. mioga* has $2n = 55$ and all others have $2n = 22$ indicating a polyploid series with multiples of eleven chromosomes. All the three species of *Zingiber* investigated in the present work have $n = 11$.

Polyploidy and aneuploidy combined with the structural changes of chromosomes were presumed to be causes for evolution of higher chromosome numbers in Scitamineae as a whole (Raghavan and Venkatasubban, 1943; Sharma and Bhattacharya, 1969; and Sato, 1960). According to these authors, 4, 5 and 6 are the original basic numbers and the reported basic number of 9, 10, 11, 12 and 13 are the secondary basic numbers derived from the primary numbers. A chromosome number of $2n = 55$ for *Z. mioga* reported by Morinaga *et al.* (1929) and Sato (1948) was interpreted by Chakravorti (1948) as a pentaploid with a basic number of $x = 11$.

Mahanty (1970) presumed a step-wise increase in basic number in the sub-family Zingiberoideae such as $x = 11$ in *Zingiber*, *Kaemferia*, 12 in *Alpineae*, 13 and 14 in *Cienkowrkya*, 16 in *Globba* and 17 in *Hedychium* and concluded that the increase or decrease of chromosome numbers from the original basic number of 11 has not occurred through simple loss or gain in chromosome, but through complete structural changes.

Multivalent association observed in the diploid species of *Zingiber* in the present investigation has been explained on the basis of structural heterozygosity for translocation. Based on the earlier reports and present investigation, a basic chromosome number of $x = 11$ has been inferred for the genus *Zingiber*.

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