Cytogenetical Studies on Ecological Races of *Mecardonia dianthera* (SW) Pennel III. Induced polyploidy

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Two diametrically opposite approaches towards the understanding of the genetic differentiation within and between various types of populations are possible. One of these is to analyze the existing natural populations and the other is to produce experimental populations and to study them under controlled conditions. While the former aspect was studied in *Mecardonia dianthera*, a Scrophulariaceous weed of Tropical America, (Kaul 1972 b), the present paper deals with an aspect of latter approach. An analysis of variation pattern within this species revealed it to comprise of two morphologically distinct ecotypes (*viz.*, repent and erect) and two ecads (normal and compact repent form, Fig. 1). In nature, the repent ecotype grows either in moist shady protected habitats or on dry hard habitats exposed to trampling and other biotic disturbances. The erect ecotype gregariously grows in moist protected habitats, cultivated fields, gardens, lawns etc, over neutral or slightly basic soils. It was found that while the erect ecotype is a facultative calcicole, the repent ecotype is strictly an obligate calcifuge (Kaul 1972 b). These ecotypes differ at genetic level as well and exhibit a monogenic difference in repent/erect character (Kaul 1969 c), and similarities in chromosome number, karyotype, pairing behaviour and chiasma frequency (Kaul 1969 b). Nevertheless, these two ecotypes were found to differ from each other in ecological distribution, phenology, habitat preference, germination potential, reproductive and aggressive capacity (Kaul 1967, 1969 a, 1972 b).

It is generally accepted that the genecological differentiation of plant populations is the result of natural selection and that the products are adapted to the particular local habitat conditions. Since the early publications of Petersen (1915) and Turesson (1922), while morphological, taxonomical and physiological aspects of genecological differentiation have received much attention (Wilkins 1960, Heslop-Harrison 1964, Ward 1969, Brerton 1971, Kaul 1972 b), studies towards the understanding of the mode of origin and evolutionary tendencies of edaphic ecotypes with a permanent inbreeding system are neglected so far. Accordingly, in order to understand the evolutionary trends in this species complex, induction of variation through chemicals and radiation treatments was tried and this paper gives an account of plant performance and cytological behaviour of colchicine induced tetraploids of *M. dianthera* ecotypes. The object of the present paper is to put on record the behaviour and differential response exhibited by ecological races to certain potent chemicals, especially presently to the colchicine, a universally accepted polyploidizing drug.

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Materials and methods

Seeds for the present studies were collected from the inbred lines of these ecotypes maintained for genetic studies by the author. The inbred line of erect ecotype was homozygous for erect gene (R) and that of repent ecotype was homozygous for repent gene (r). 100 seeds of each of these ecotypes in 5 replicates were presoaked for 12 hours in distilled water and four replicates of each ecotype were treated with 0.3% of colchicine solution for 12 hours. The treated seeds were washed thoroughly in water and germinated in a germinator. In all 63 and 56 surviving seedlings respectively of erect and repent ecotype emerged from the treated seeds and these were transplanted in pots. Out of these 24 and 18 plants respectively of erect and repent ecotype were found to be tetraploids with $2n=44$ and with morphological traits as tabulated in Tables 1-2. Others looked normal and resembled the control set maintained along with this experiment.

Crossing and cytological studies were done by the methods outlined earlier (Kaul 1969 b, c). For statistical parameters, methods given by Woolf (1968) were followed. Bartlett's test was applied for determining the homogeneity of variance in various chromosome associations among the PMCs of the colchiploids of an ecotype and this test gave nonsignificant χ^2 values, hence the data from all the colchiploids of an ecotype was pooled and transformed into one analysis. However, the test indicated significant χ^2 values with regard to univalents and laggards (Table 5).

Observations

Morphology and plant performance: In general the tetraploids of both the ecotypes exhibited slower growth rate; delayed phenological events; deep green thick, rough foliage; increased shoot-root dry weight; enlarged epidermal and mesophyll cells, chloroplasts, stomatal size and frequency and pollen grains than that of diploid control sets (Tables 1, 2). In particular the tetraploid erect plants had significantly greater shoot length, plant weight than that of the diploids. The deep green leaf colour of the tetraploids was found to be due to the presence of more numerous and larger chloroplasts than those of the diploids. The midrib and veins of the leaves of the tetraploids were increased in size. A transverse section revealed larger and broader epidermal cells in the tetraploids than in the diploids of the similar age group. The spongy cells were of enlarged size in the tetraploids. The flowering though sparse in the tetraploids extended longer than that in the diploids. The seed setting of the tetraploids was poor and most of the fruits were either abortive or with shrivelled embryos. Few seeds that were produced by the tetraploids developed a rudimentary radicle upon germination that considerably swelled and finally perished. The plumule in these never emerged and hence seedlings from these could not be recovered.

While the polyploids exhibited a morphological gigas, the root length, leaf dimensions and stomatal index between the erect diploids and its induced tetraploids differed insignificantly (Table 2). Nevertheless, there was a significant reduction in floral number in the erect tetraploids in comparison to its diploid progenitors. On the other hand, the tetraploids repent plants in particular exhibited significant in-

crease in aboveground plant biomass, underground root dry weight, leaf length, stomatal index and floral and fruit number per plant.

Cytology: Leaf tip counts of the polyploids revealed $2n=44$ in both the ecotypes but a few cells with chromosome number ranging from 38 to 46 were also observed. While meiotic events of the diploid plants of *M. dianthera* were normal

Table 1. Plant performance of repent ecotype of *M. dianthera*
(Mean and standard deviation values)

Plant character	Diploid	Tetraploid
Shoot: maximum spread (cm)**	28.6 $\pm$ 2.1	64.7 $\pm$ 1.4
branch number per plant	8.3 $\pm$ 2.2	31.4 $\pm$ 3.1
length of the longest branch**	16.54 $\pm$ 1.62	29.83 $\pm$ 4.63
fresh weight (gm)**	3.39 $\pm$ 0.42	8.24 $\pm$ 0.97
dry weight (gm)**	0.653 $\pm$ 0.13	1.194 $\pm$ 0.53
Root: length (cm)	12.32 $\pm$ 1.44	11.93 $\pm$ 1.15
fresh weight (gm)	0.483 $\pm$ 0.68	0.512 $\pm$ 0.074
dry weight (gm)*	0.092 $\pm$ 0.013	0.125 $\pm$ 0.08
Total dry matter production (biomass)	0.745	1.319
Leaf: length (cm)	1.78 $\pm$ 0.12	1.97 $\pm$ 0.10
breadth (cm)	0.51 $\pm$ 0.16	0.62 $\pm$ 0.13
length/breadth	3.4	3.17
Stomatal apparatus:		
size of the guard cells (μ)*	29.6 $\times$ 21.2	37.5 $\times$ 23.8
	$\pm$ 1.9 $\pm$ 2.2	$\pm$ 2.1 $\pm$ 2.6
size of the aperture (μ)	10.2 $\times$ 6.5	12.7 $\times$ 7.1
	$\pm$ 1.3 $\pm$ 0.8	$\pm$ 3.4 $\pm$ 2.2
fruequency on dorsal surface (leaf)**	240 $\pm$ 5.4	156 $\pm$ 4.3
frequency on ventral surface (leaf)**	163 $\pm$ 8.6	91 $\pm$ 4.9
index of the dorsal surface	37.6 $\pm$ 1.8	36.5 $\pm$ 1.7
index of the ventral surface	29.1 $\pm$ 1.9	27.8 $\pm$ 1.7
Pollen grains:		
fertility percent**	90.6 $\pm$ 3.5	14.3 $\pm$ 2.7
size in μ , length*	20.2 $\pm$ 0.6	33.4 $\pm$ 9.8
breadth	18.5 $\pm$ 0.7	31.2 $\pm$ 11.5
pooled mean	19.33 $\pm$	32.36
Floral number per plant**	20.4 $\pm$ 3.2	13.3 $\pm$ 2.4
Seed germination percent**	51.5 $\pm$ 5.7	10.2 $\pm$ 3.4
Seedling survival percent**	38.7 $\pm$ 4.3	nil

$\pm$ indicates standard deviation value, * $P < 0.05$, ** $P < 0.01$.

and regular, these in colchipooids were abnormal and of similar nature in the plants of both the ecotypes; hence a common meiotic account of these colchipooids is given in the following.

Early prophase stages in the material could not be properly analyzed as the chromosomes were clustered into irregularly limited configurations of erratic nature.

The nuclei of 3–8% PMCs were observed in the synzesis, and during pachytene the pycnotic degeneration expressed in the form of minute heteropycnotic nodules along the single chromosomal fibre. In a few such PMCs, the pycnosis of an advanced stage was also observed. The pycnotic degeneration in pachytene resulted in the formation of chromatin stickiness at MI (Figs. 5, 6).

Observations on chromosomal association were recorded mostly at late diaki-

Table 2. Plant performance of erect ecotype of *M. dianthera*
(Mean and standard deviation values)

Plant character	Diploid	Tetraploid
Shoot: height (cm)*	13.6 ± 1.23	19.85 ± 2.17
fresh weight (gm)*	1.46 ± 0.22	2.03 ± 0.56
dry weight (gm)*	0.238 ± 0.035	0.482 ± 0.17
Root: length (cm)	8.53 ± 1.22	9.88 ± 2.70
fresh weight (gm)*	0.23 ± 0.02	0.34 ± 0.05
dry weight (gm)*	0.065 ± 0.018	0.091 ± 0.042
Total dry matter production (biomass)*	0.303	0.573
Leaf: length (cm)	1.5 ± 0.22	1.7 ± 0.30
breadth (cm)	0.7 ± 0.13	0.7 ± 0.15
length/breadth	2.1	2.4
Stomatal apparatus:		
size of the guard cells (μ)*	29.8 × 20.9	36.3 × 24.6
	±1.5 ± 1.8	±2.7 ± 2.2
size of the aperture (μ)	10.6 × 6.2	13.5 × 7.4
	±0.7 ± 0.9	±2.8 ± 1.9
frequency on dorsal surface (leaf)*	223 ± 7.6	141 ± 5.8
frequency on ventral surface (leaf)*	226 ± 9.81	107 ± 7.2
index of the dorsal surface	36.4 ± 1.44	32.32 ± 3.85
index of the ventral surface	35.7 ± 1.30	33.94 ± 2.68
Pollen grains:		
fertility percent**	93.6 ± 4.8	12.3 ± 2.9
size in μ, length*	20.4 ± 1.3	31.3 ± 8.8
breadth*	18.3 ± 2.44	26.4 ± 9.4
pooled mean	19.36	28.88
Floral number per plant*	9.7 ± 1.62	5.1 ± 1.4
Seed germination percent**	57.6 ± 3.84	5.5 ± 2.43
Seedling survival percent**	43.4 ± 3.9	Nil

± indicates standard deviation value, *P < 0.05, **P < 0.01.

nesis and early metaphase stages since chromosomes were contracted and sometimes clumped together at late MI in many PMCs. However, the frequency of univalents could be determined with certainty at late diakinesis as well because these remained nonaligned and distant to the congressed chromosomal associations. In many sporocytes a tendency of chromosome associations to lie close to each other in groups of 2–3 was also observed. In many of these it was difficult to decide whether a con-

figuration comprised of more than 2 to 3 chromosomes associated as a multivalent or of the bivalent or trivalent lying so close to the other uni, bi, or tri-valents; occasionally these were too close and appeared almost to touch each other. Probably these

Figs. 1-3. 1, three forms of *M. dianthera*, A, erect form. B, normal repent form. C, compact repent form. 2, erect form in culture. 3, repent form (4X). Magnification (approximate) Fig. 1 $\times 1/2.5$, Fig. 2 $\times 1/4$, Fig. 3 $\times 1/5$,

represented an expression of secondary association between various chromosomal associations. While extremely doubtful cases were deleted, cases with some certainty are recorded in parenthesis in Tables 3 and 4.

Varying number of chromosomal associations ranging from bivalents to hexava-

Figs. 4-10. 4, metaphase I with uni to hexavalent associations. 5, clumped chromosomes forming 2 compact metaphase plates. 6, compact metaphase I plates exhibiting an anaphasic stretch. 7, early anaphase I. 8-9, late anaphase I with laggards. Fig. 10, telophase I exhibiting unequal chromosome distribution. Figs. 4, 5, 10, $\times 3,600$. Figs. 7-9, $\times 3,950$.

lents were observed at diakinesis and MI (Tables 3, 4, Fig. 15), but clearcut and sharply defined pentavalents were rarely encountered. Among multivalents, quadrivalents occur in maximum and hexavalents in minimum frequency in both the colchipooid types (Tables 3, 4). The total number of multivalents nearly equalled the number of univalents on an average per PMC per ecotype (Fig. 15). However, the colchipooids of repent ecotypes possessed more bivalents and less uni and multivalent associations than those of erect ecotype. In 12 to 18% PMCs, MI was characterised by sluggish movement of chromosomes resulting in inceptant MI plates. In 16 to 35% PMCs, the chromosomes exhibited adhesions and stickiness and got arranged into 2 to 3 metaphase plates, one at right angles to the other (Fig. 5), each plate

Figs. 11-14. 11-12, prophase II with macro and micro nuclei. 13, metaphase II with compact chromosomal plates. 14, anaphase II exhibiting anaphasic stretch and micronuclei. Magnification (approximate): Figs. 11-14 $\times 3,950$.

organising its own spindle. In 24 to 39% PMCs, clumping of chromosomes on the equatorial plate was observed and hence such chromosomal associations could not be analyzed. Further divisions in these revealed their independent functioning. This aberrancy involved cytoplasmic irregularity and a consequent failure of cytokinesis resulting in mixing of some chromosomes of one plate with those of the other. The univalents at MI lie at random, situated either at the equator or deviated towards a polar end (Fig. 4). That these univalents are not due to precocious disjunction of the homologues is evident by their existence as such without any association at all

at the late diplotene in some PMCs. Within the colchipooids, heterogeneity among the frequency of univalents per PMC was significant (Table 5).

Anaphasic separation was asynchronous and the distribution of the chromosomes was random and irregular (Figs. 7-9). Variable number of lagging chromosomes single or in associations were observed in 72-87% PMCs (Figs. 8, 9). These laggards either reached the poles or lagged to remain at the centre as micronuclei (Figs. 8, 9). Among the colchipooids of an ecotype, the heterogeneity chi-square values among the PMCs in univalent frequency was significant (Table 5). 11 to 24%

Table 3. Proportion of various chromosomal associations in the repent colchipooid of *M. dianthera*

Chromosomal association at metaphase I	Frequency
1VI + 5IV + 4II + 10I	16
2VI + 3IV + 4II + 12I	5
2VI + 6IV + 4II	9
3VI + 3IV + 1III + 3II + 5I	8
5IV + 2III + (4II + 4I) + 6I	27
5IV + (2III + 2I) + 2II + 12I	14
6IV + 2III + 3II + 8I	48
6IV + 2III + 4II + 6I	36
6IV + 3III + 1II + 9I	20
Unclassified and miscellaneous	17

Table 4. Proportion of various chromosomal associations in the erect colchipooid of *M. dianthera*

Chromosomal associations at metaphase I	Frequency
1VI + 5IV + 3III + 9I	9
2VI + 4IV + 3III + 7I	8
4IV + (5III + 3I) + 1II + 8I	24
5IV + 3III + (4II + 2I) + 5I	38
5IV + 4III + 2II + 8I	35
5IV + (3III + 5I) + 10I	29
6IV + (3III + 2I) + 9I	26
6IV + 2III + 1II + 12I	17
Unclassified and miscellaneous	16

PMCs were characterized by an absence of active polar movement (Fig. 7). This resulted in the formation of restitution nuclei in these, that developed into a large sized pollen grains which could never be germinated *in vitro* by the author. At telophase I, and uneven number of chromosomes occur at the poles and some laggards get excluded from the main nuclei and these later tend to form the micronuclei (Fig. 10).

During interkinesis, the chromosomes lie scattered and despiralized around the nucleus. Laggards form micronuclei (Figs. 11, 12) and some among these appear

to have fused with the macronucleus resulting in club shaped extensions of the interkinetic nodules (Fig. 11). No discernible metaphase plates at MII were observed, instead the chromosomes were condensed and fused together over the incept metaphase plates (Fig. 13). In 33 to 44 % PMCs, loose grouping of the chromosomes suggesting an evidence of failure of spindle movement rather than complete break-

Fig. 15 Total proportionate number of multivalents (A), univalents (B) and bivalents (C) in the two colchipooids.

Table 5. Number of univlants, laggards and micronuclei during the microsporogenesis of *M. dianthera* colchipooids

Plant type	Metaphase I (univalents)		Anaphase I (laggards)		Anaphase II (laggards)		Tetrads (micro-nuclei)	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Erect ecotype	2-11	7	4-13	10	5-16	8	1-4	2
Heterogeneity χ^2	71.8*		75.2*		76.1*		37.8	
Repent ecotype	2-12	6	3-10	11	6-15	9	2-5	3
Heterogeneity χ^2	68.2*		71.4*		73.7*		43.6	

* $P < 0.05$, $df. = 49$.

down of cell division mechanism was observed. Polar movement of chromosomes was not observed in 17 to 36 % PMCs thus indicating a breakdown of cell division mechanism in these. While in a few (13-18 %) anaphasic stretch was observed, in others anaphasic movement of chromosomes took place but in these presence of laggards was invariably found (Fig. 14).

At telophase II, more than 2 micronuclei and monads to pentads of different shape, size and configuration were observed. Because of the abnormal meiotic events, the microspore nuclei had varying number of chromosomes. Microspores with higher chromosome number were larger in size than those with lesser number. While a few young pollen grains were shrunken, majority of the mature grains at dehiscence were large and deformed in shape and only 10 to 17% stained with aceto-carmin stain.

Crossing: Only 5–7% pollen grains could be germinated on the stigma of the other colchipooids but seed set in these artificially crossed flowers never took place. Instead, the ovary in these degenerated prematurely. On the other hand, natural self pollination produced 5–8% seeds that upon germination extruded a radicle but never germinated fully to develop into a seedling. All direct and reciprocal crossings between the tetraploids resulted in no seed set in the colchipooids.

Discussion

Biological systems express and reveal their specificity in different ways and at different levels. The specificity to the drug colchicine is manifested by the two edaphic ecotypes of *M. dianthera* in which cochicine induced tetraploidy to a differential degree resulting in a morphological gigas, increased dry matter production and seed sterility. While repent ecotype tetraploids had increased flower number per plant than that of the diploids, reverse was observed in the erect ecotype tetraploids. Also more number of erect surviving tetraploids could be recovered than those of the repent ecotypes. This can be attributed to a differential diplontic selection operating in the cells of the two ecotypes. However, the colchipooids of both the ecotypes resemble other usual induced polyploids in exhibiting a slower growth rate, delayed phenological events, deep green thick rough foliage, increased dry matter production, enlarged epidermal and mesophyll cells, chloroplasts stomatal size and frequency and pollen grains than that of their diploid progenitors.

The type and frequency of chromosomal associations depends upon the degree of chromosomal pairing at zygotene and the frequency and distribution of chiasmata at pachytene (Hazarika and Rees 1967, John and Lewis 1968). But the pairing and chiasma formation are under the control of genetic systems as well (Rees 1961, Riley and Law 1965). In the present material the genotypic control seems to be expressed since a significant difference between the two colchipooids of the ecotypes with regard to frequency of multivalents and univalents was observed.

It is well known that the distribution of different kinds of chromosomal configurations (multi, bi, and uni-valents) at first metaphase of meiosis in autotetraploids depends upon the frequency and location of chiasmata between homologous chromosomes. Durrant (1960) derived formulae which predict frequency of various types of configurations with varying chiasma frequencies. One of the assumption of the model upon which these theoretical predictions were based was that a random association involving all four chromosomes of each homologous set was obligatory at pachytene. The same assumption underlines a latter model put forward by John and Henderson (1962). Accordingly these colchipooids of *M. dianthera* could form

a theoretical maximum of 11 quadrivalents. Instead, the observed quadrivalent frequency was much lower than expected (Tables 3, 4). Although a significant excess of quadrivalents over the bivalents, indicating that the association between the arms of homologous chromosomes was random was observed, the data on the chromosome associations indicate that pachytene association does not invariably involve all the chromosomes of the homologous set. This restriction contributes to the observed frequency of hexa, tri, bi and uni-valents at the expense of tetravalents (Tables 3, 4, Fig. 15). However, the strong competition of pairing between homologous chromosomes of a complement also accounts for the observed associations other than quadrivalents and the observed univalents represent probably those chromosomes that failed to get a pairing partner or a homologous chromosomal segment of length sufficient for an effective pairing.

The occurrence of chromosomal associations, especially the bivalents in pairs observed at diakinesis and MI in the present material, could be ascribed to the occurrence of residual attraction between genetically similar chromosomes. However, Riley (1960) and Kempana and Riley (1964) have indicated that the association of bivalents in pairs at MI may be a relic of prophase attraction rather than a secondary attraction of bivalent during diakinesis and MI.

Multipolar divisions reported rarely from natural diploid populations (Tai 1970) occur in many hybrids and polyploids (Kaul 1964, 1965 a, 1973, Tai 1970). Plants with meiotic irregularities due to polyploidy and wide hybridization appear to be especially susceptible to multipolar divisions (Kaul 1964, 1965 b, Tai 1970). That the spindle organizer is a single organelle which is genome specific and functions like a centriole of an animal cell could be used to explain the multiple genome separation in polyploids. Usually spindle organizer is a single unit cell organelle that gets broken by chemicals (Kihlman 1966), irradiations (Puza and Srb 1964), low temperature (Huskins and Cheng 1950) and sometimes spontaneously (Tai 1970). A random break induced in the spindle organizer would lead to a random chromosome grouping as observed presently and earlier also (Kaul 1973). Probably the number of chromosomes attracted may be proportional to the size of the broken piece of spindle organizer.

Cytokinesis seems to occur as an interaction between two spindle organizers and no genome specificity is observed in this regard. Therefore, wherever there is a spindle organizer, cytokinesis could take place, cleaving the cytoplasm and keeping the constant ration of spindle organizer per each daughter cell. Thus as the number of spindle organizer increases, then number of cytokinesis should also increase with it producing more micronuclei. However, such a relationship between spindle poles and the division furrow could not be observed in the present material. Nevertheless, the formation and behaviour of multipolar spindle have a genetic significance. Thompson (1963) and Kaul (1965 a, b) suggested that multipolar divisions and complement fractionation provide a method to decrease the level of ploidy. Hence an intrinsic tendency towards reversion to diploidy appears in both the colchiploids of *M. dianthera*.

A little uncertainty appears about the observed data upon which the correlation coefficient values are based. Because a few doubtful cases such as indicated in pa-

renthesis in Tables 3, 4 had to be included in the calculations since these could not be dispensed with in the random sampling method followed while making cytological observations presently. Hence although a significant positive correlation between univalents at diakinesis and at MI was observed (Table 6), a relatively greater number of univalents at MI than at diakinesis were detected. Also, that chiasmata within some chromosomal associations had terminalized before they were attached to the spindle could also lead to the formation of a greater number of univalents at MI than at diakinesis. Nevertheless, the multivalents with terminalized chiasmata have consequently greater flexibility permitting a more orderly orientation on the equatorial plate. Significant positive correlation between chiasma frequency and multivalent frequency indicates that the chiasma number per association increased with increasing multivalent formation.

A close positive correlation between the frequency of MI cells with univalents at MI represent laggards at AI. Further, the lagging univalents at AI seem to represent

Table 6. Correlation coefficient values between some meiotic events in *M. dianthera* colchipooids

Correlation coefficient value (r) between:	Plant form	
	Erect	Repent
a) univalents at diakinesis and univalents at MI	+0.42*	+0.46*
b) univalents at MI and laggards at AI	+0.53*	+0.61*
c) univalents at AI and lagging chromatids at AII	+0.56*	+0.62*
d) univalents at AI and micronuclei in tetrads	+0.32	+0.27
e) lagging chromatids at AII and micronuclei in tetrads.	+0.69**	+0.65**
f) univalents at AI and pollen sterility	+0.32	+0.40
g) lagging chromatids at AII and pollen sterility	+0.48*	+0.43*
h) chiasma frequency and univalents at MI	-0.19	-0.34
i) chiasma frequency and univalents at diakinesis	-0.28	-0.37
j) chiasma frequency and multivalents at MI	+0.47*	+0.51*

* $P < 0.05$, ** $P < 0.01$, df.=23.

the lagging chromatids at AII ($r = .63, .70, P < .01$), which in turn represent the micronuclei in tetrads ($r = .65, .69, P < .01$). While Nath and Nielson (1962) and Opsahl (1964) reported a close relationship between lagging univalents at AI and pollen stainability in timothy, such could not be observed in the present material (Table, 6). Further, partialling out the influence of the factor 'number of laggards at AII', an extremely weak insignificant positive correlation ($r = .073$) between the number of laggards at AI and the pollen sterility was observed. Also when the factor 'number of laggards at AI' was kept as the dependent variable and the laggards at MII and pollen sterility as the independent variables, coefficient of multiple determination (R^2) was also insignificant ($R = .31$). Hence the contribution of factors other than univalents especially of the multivalents, than equal the univalents in number, in inducing the pollen sterility in the present material appears quite significant.

Conclusion: The present study indicates the two ecological races exhibit a

differential susceptibility to colchicine in relation to the i) frequency of induced polyploids, ii) morphological and reproductive features, iii) number and frequency of chromosomal associations and univalents, iv) pollen and seed sterility. However, a general agreement in having a morphological gigas, slowed growth rate, delayed phenological events and reduced reproductive capacity occurs in the tetraploids of both the ecotypes. A tendency to change the plant form (repent/erect) which is governed by a single gene, was not observed, indicating that the gene remains unaffected by the drug, in both the ecological races of *M. dianthera*.

Summary

Mecardonia dianthera, a Scrophulariaceous weed of Tropical America comprises of two edaphic ecotypes viz. erect and repent. While the former is a facultative calcicole, the latter is an obligate calcifuge. The present paper gives an account of plant performance and cytological behaviour of colchicine induced tetraploids that were produced by treating the inbred lines of these plants with 0.3% colchicine for 12 hours. Majority of the surviving plants were tetraploids exhibiting a slower growth delayed phenological events, deep green thick green rough foliage, increased dry matter production, enlarged epidermal and mesophyll cells, chloroplast, stomatal apparatus and frequency, pollen grains and a reduced seed viability than that of the diploids. While repent ecotype tetraploids had increased flower number per plant than that of the diploids, reverse was observed in the erect ecotype tetraploids.

At metaphase I, uni, bi, tri, tetra and hexa-valents in a varying frequency were observed in the colchipooids and the total number of multivalent associations and univalents per PMC on an average was equal in the tetraploids of each ecotype. This indicates a competition for pairing between homologous chromosomes which is supported by the fact that a significant positive correlation between chiasma frequency and multivalent formation was also observed. However, the colchipooids of the erect ecotype possessed greater number of multivalents and univalents than those of the repent ecotype colchipooids. Further stages of meiosis in the colchipooids were abnormal and resembled the typical autoploid meiosis. Multiple spindles, complement fractionation and secondary association were also observed. The contribution of univalents and multivalents in inducing pollen sterility in the tetraploid material is significant. Attempts to cross these colchipooids with diploids produced non-viable abortive seeds. Artificial crossings amongst these colchipooids resulted in an early degeneration of the ovary and a failure of the seed formation. Natural self pollination yielded 5-9% of seeds that died after an extrusion of radicle when germinated.

The study indicated that the two edaphic ecological races (ecotypes) exhibit a differential susceptibility to colchicine in relation to frequency of induced polyploids, morphological and reproductive features, number and frequency of chromosomal associations and univalents, pollen and seed fertility. However, a general agreement in having a morphological gigas, slowed growth rate, delayed phenological events and reduced reproductive capacity occurs in the tetraploids in comparison to the diploid progenitors, of both the ecotypes. A tendency to change the plant form (repent

erect), which is governed by a single gene, was not observed, indicating thereby that the gene remains unaffected by the drug, in both the ecological races of *M. dianthera*.

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