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STUDIES IN COLCHICINE-INDUCED TETRAPLOID OF *ATYLOSIA SCARABAEOIDES*[1]

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ABSTRACT

Autotetraploids of *Atylosia scarabaeoides* were raised artificially by colchicine treatment. Tetraploid showed giganticism in many vegetative and reproductive characters. Many PMCs showed univalents, trivalents and quadrivalents besides bivalents at metaphase I. Very few PMCs had all bivalents only. 22% PMCs showed unequal distribution of chromosomes, laggards etc at anaphase I and the remaining 78% PMCs had normal and regular separation of chromosomes. Slight decrease in pollen fertility was observed in tetraploids and it resulted in almost normal seed setting and fruit formation.

INTRODUCTION

The effects of induced polyploidy are various and they may have economi cally desirable characters also. They also help in understanding the evolutionary mechanism by comparing the morpho logy, breeding behaviour and cytolotical features of induced polyploids with those of their diploid counterparts. With this aim, induction of polyploidy by colchicine treatment in *Atylosia scarabaeoides*, an economic plant of considerable importance, has been taken up by the authors. No Natural or artificial tetraploid of this species is known as yet. This induced tetraploid when crossed with other tetraploids and higher polyploids of *Atylosia* species may help in determining their phylogenetic relationship. By crossing the induced tetraploid with its normal diploid plant it may further help in obtaining triploid and aneuploids which may be economically improved plant.

MATERIAL AND METHODS

5-6 days old seedlings of *Atylosia scarabaeoides* were subjected to apical bud treatment with aqueous colchicine solutions (0.1%, 0.2%, 0.3%, 0.4%, 0.5%). Apical buds were covered with absorbent cotton soaked with colchicine solutions -8 hours a day for one, two and three days. Hours of treatment were from 7.30 a.m. to 3.30 p.m. to synchronise with the time of division of apical cells. After eight hours of treatment soaked cotton was removed from apical buds and they were washed thoroughly with water. Morphological and cytological studies were made in treated plants. Cytological studies were made by root tip and young anther squashes stained with acetocarmine.

OBSERVATION

0.2% and 0.3% colchicine solutions were observed to be effective in producing polyploid shoots. Out of 180 plants

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treated with above solutions, 21 tetraploids were induced and only 3 could survive till maturity. The rest 18 plants died due to heavy infection of fungi (Powdery mildew). The effect of colchicine treatment was evaluated by making morphological and cytological investigations.

Morphological studies—Morphological characters of both control (diploid) and induced tetraploids were very carefully observed and their comparative accounts and measurements of various parts have been summarised in Tables I and II.

Cytological studies—Anther squash of induced tetraploids showed n =22 in PMCs at diakinesis and metaphase I having various associations and configurations of chromosomes (Figs. 7, 8 and 9) (Table III). The range and mean of univalents, bivalents, trivalents and quadrivalents were also determined by analysing PMCs at metaphase I. A comparative account of chromosome pairing and chiasma frequency at metaphase I of diploids and induced tetraploids have been summarised in Table—IV.

At anaphase I regular and normal separation of 22-22 chromosomes was observed in 78% PMCs while remaining 22% PMCs showed unequal separation of chromosomes and laggards (Table V). Leggards were also observed in some PMCs at anaphase II. The size of pollen grains increased in induced raploids but their fertility decreased (Tables II and III).

DISCUSSION

The production of polyploids experimentally by colchicine treatment provides an opportunity to assess their importance in the improvement of yield of crops and some other characters. The review by Noggle (1946) and survey of literatures on polyploidy with increased instances of induced tetraploids reveal that gigantism is not always an attending feature of polyploidy. In the present study colchicine induced tetraploids were found to be superior to their diploid progenitor in height and size of leaves. Height of a tetraploid does not increase only by elongation of internodes, rather the number of nodes is also responsible for it. It is therefore quite natural that the number of secondary branches and inflorescences would also increase due to formation of more nodes.

The poor reproductive performances of induced tetraploids versus diploids as regards the size and number of seeds and fruits may be explained due to a number of cytological situations that are found in tetraploids and absent in diploids. The various types of quadrivalent configuration and their irregular seggregation lead to formation of unbalanced gametes resulting in poor fertility. Due to these cytological irregularities induced tetraploids were found to be inferior to diploids in the present investigation as observed in some cucurbits and many other plants.

The death of tetraploid plants by infection shows that polyploidy does not confer disease resistance to this species although Rajan (1961) reported the case of aphid resistance to be associated with autopolyploidy in *Brassica compestris*.

A perusal of the frequency of chromosome configuration in the induced tetraploid of *A. scarabasoides* shows the occurrence of multivalents together with univalents and bivalents in 90% PMCs. This higher frequency of cells showing multivalent formation is characteristic of autopolyploids. The observation of Morrison and Rajhathy (1960) that the mulivalent formation is higher in plants with smaller chromosomes than with longer chromosomes is in keeping with the finding of the authors as the chromosomes of *A. scarabaeoides* are very small. However,

TABLE I

MEASUREMENTS OF VARIOUS PARTS OF THE DIPLOID AND INDUCED TETRAPLOIDS-*Atylosia scarabaeoides*

Characters	Diploid		Tetraploid	
	Mean	S.E.±	Mean	S.E.±
Leaf :				
Length of the petiole (cm)	2.5	0.02	3.9	0.04
Length of the terminal leaflet (cm)	4.2	0.38	6.2	0.42
Breadth of the terminal leaflet (cm)	2.4	0.41	3.7	0.60
Length of lateral leaflets (cm)	3.5	0.52	4.3	0.66
Breadth of laertal leaflets (cm)	1.9	0.24	2.8	0.38
Thickness of the leaf (μm)	321		456	
Average number of hairs on the leaf (cm^2)	49	0.62	92	0.66
Stomata :				
No. of stomata per field (10×15)	24.9	0.12	12.8	0.18
Length of the guard cells (μm)	20.6		31.2	
Breadth of the guard cell (μm)	7.2		10.9	
Length of stomatal aperture (μm)	10.8		13.7	
Breadth of the stomatal aperture (μm)	8.6		11.9	
Flower :				
Length of the pedicel (mm)	6.2	0.58	8.8	0.42
Thickness of the pedicel (mm)	0.8	0.60	1.1	0.54
Length of the calyx tube (mm)	6.1	0.48	8.7	0.42
Breadth of the calyx tube (mm)	1.4	0.38	2.2	0.26
Length of the corolla (mm)	8.4	0.60	10.9	0.54
Standard	8.4	0.60	10.9	0.54
Wings	7.6	0.58	9.2	0.46
Keel	6.4	0.52	7.2	0.38
Breadth of the corolla (mm)	4.6	0.46	5.8	0.28
Standard	4.6	0.46	5.8	0.28
Wings	2.8	0.56	3.4	0.46
Keel	1.8	0.64	2.2	0.52
Length of the anther (mm)	0.5	0.24	0.8	0.32
Thickness of the anther (μm)	1.8		2.2	
Diameter of the pollen (μm)	30.4		42.9	
Pollen fertility	98.2%		83.8%	
Length of the ovary (mm)	4.8	0.38	5.2	0.42
Thickness of the ovary (mm)	3.6	0.38	4.2	0.42
Fruit :				
Length of the pod (cm)	2.5	0.30	1.6	0.38
Thickness of the pod (cm)	0.5	0.20	0.6	0.02
Seed :				
No. of seeds per fruit	5.8		2.9	
Size of seed (mm × mm)	2.0×1.4		2.4×1.6	
Thickness (mm)	1.2		1.4	
Weight of 100 seeds (gms)	1.232		1.604	

TABLE II

MORPHOLOGICAL DIFFERENCES BETWEEN DIPLOID AND INDUCED TETRAPLOID

Characters	Diploid	Tetraploid
Plant	Vigorous	Less vigorous in the beginning but more vigorous later on.
Stem	Profusely branched, thinner stem, long internodes.	Comparatively less branched, thicker stem, shorter internodes.
Leaves	Petiolate, thin petiole, greenish with prominent vein and veinlets, margin almost entire	Petiolate, comparatively thicker and longer petiole, dark green with more prominent veins and veinlets and have a tendency of serration on the margin.
Stomata	Small	Big (about 1.5 times the diploid)
Hairs	Leaf surface rough due to large no. of minute hairs.	Leaf surface more rough as it is covered with comparatively long and dense minute hairs.
Flower	Small and yellow flowering-Aug. -Dec.	Big and yellow, flowering delayed by one month (Sept.- Jan.)
	Different parts of the flower comparatively smaller in size.	Different parts bigger in size.
Pollen :		
Size	Small	Big
Fertility	98.2%	83.8%
Fruit	Comparatively bigger (with seeds ranging from 5-7) with an average of about 6 seeds in each pod.	Smaller & thicker (with seeds ranging from 2-4) with an average of about 3 seeds in each pod.

TABLE III

FREQUENCY OF TYPES OF CHROMOSOME CONFIGURATIONS IN THE INDUCED TETRAPLOID *A. Scarbaeoides* AT METAPHASE I.

Chromosome configuration	No. of PMCs	Percentage
$^{0}I + {}^{22}II$	5	10%
$^{1}I + {}^{20}II + {}^{1}III$	1	2%
$^{0}I + {}^{20}II + {}^{0}III + {}^{1}IV$	6	12%
$^{1}I + {}^{18}II + {}^{1}III + {}^{1}IV$	1	2%
$^{0}I + {}^{18}II + {}^{0}III + {}^{2}IV$	14	28%
$^{0}I + {}^{16}II + {}^{0}III + {}^{3}IV$	15	30%
$^{1}I + {}^{14}II + {}^{1}III + {}^{3}IV$	4	8%
$^{0}I + {}^{14}II + {}^{0}III + {}^{4}IV$	3	6%
$^{0}I + {}^{12}II + {}^{0}III + {}^{5}IV$	1	2%

TABLE

CHROMOSOME PAIRING AND CHIASMA FREQUENCY IN THE

Name of the species	No. of PMC analysed	No. of Univalents			No. of bivalents per PMC						No. of triva per PMC	
		Range	Mean	S.E.±	Rod			Ring			Range	Mean
					Range	Mean	S.E.±	Range	Mean	S.E.±		
1	2	3	4	5	6	7	8	9	10	11	12	13
Diploid	40	—	—	—	3—7	5.15	0.22	4—8	5.85	0.28	—	—
Induced tetraploid	50	0—1	0.12	0.02	0—4	1.32	0.03	12—22	16.28	0.25	0—1	0.12

due to presence of four homologous chromosomes, multivalent association is expected in all PMCs of autotetraploid. But the formation of only bivalents in 10% PMCs (Table III, Fig. 9.) suggests that the presence of more than two homologous chromosomes is not the only requisite for multivalent formation. The frequency of multivalents was considerably lower (Table IV) and no association of more than 4 chromosomes was observed. Hence, on the basis of these findings, it can be suggested that there must be a genetic control so far as chromosome pairing is concerned. Such reports are available in the literature (Riley and Chapman, 1958). Perfect bivalent formation as occurs in normal sexual species is the apical stage in the evolutionary adaptiveness of the species. Duplication of chromosome set at one stage creates a novel situation with the result that in the limited time and space, four homologous sets are not able to behave as normally as two sets do. Moreover, genetic control of chromosome pairing has also been observed in colchicine induced diploids of haploid *Pelargonium* (Daker, 1967). Induced tetraploids display a series of cytological irregularities at meiosis leading ultimately to reproductive deficiency.

The distribution of chromosomes at anaphase is disturbed more by univalents and trivalents than quadrivalents due to unequal assortment and laggards. This is proved by the fact that 78% PMCs had quadrivalents in different number together with bivalents at metaphase I and regular and equal distribution of chromosomes was also found in 78% PMCs at anaphase I. Similarly univalents and trivalents were found to occur in 22% PMCs at metaphase I and unequal distribution of chromosomes with or without laggards was also found in 22% PMCs. Thus it can be safely concluded that frequency of univalents and trivalents at metaphase I may be related with anaphasic irregularities of meiosis. As a result of unbalanced chromosome number arising due to above irregularities autotetraploids have some pollen sterility. Only those pollen grains receiving haploid or diploid sets of chromosomes are likely to survive and the remaining with unbalanced number are sterile pollen grains.

Authors find the mean value of fertility in induced tetraploids to be 83.8%

IV

DIPLOID AND INDUCED TETRAPLOID AT METAPHASE I

lents	No. of quadrivalent per PMC			Total $2n$	Chiasmata per PMC			Terminalised chiasmata per PMC			Terminalisation co-efficient	½ chiasma per chromosme
S.E. ±	Range	Mean	S.E. ±		Range	Mean	S.E. ±	Range	Mean	S.E. ±		
14	15	16	17	18	19	20	21	22	23	24	25	26
—	—	—	—	22	15—21	17.67	0.12	14—19	16.85	0.12	0.953	0.803
0.02	0—5	2.18	0.16	44	36—44	42.64	0.32	32—40	38.64	0.32	0.906	0.969

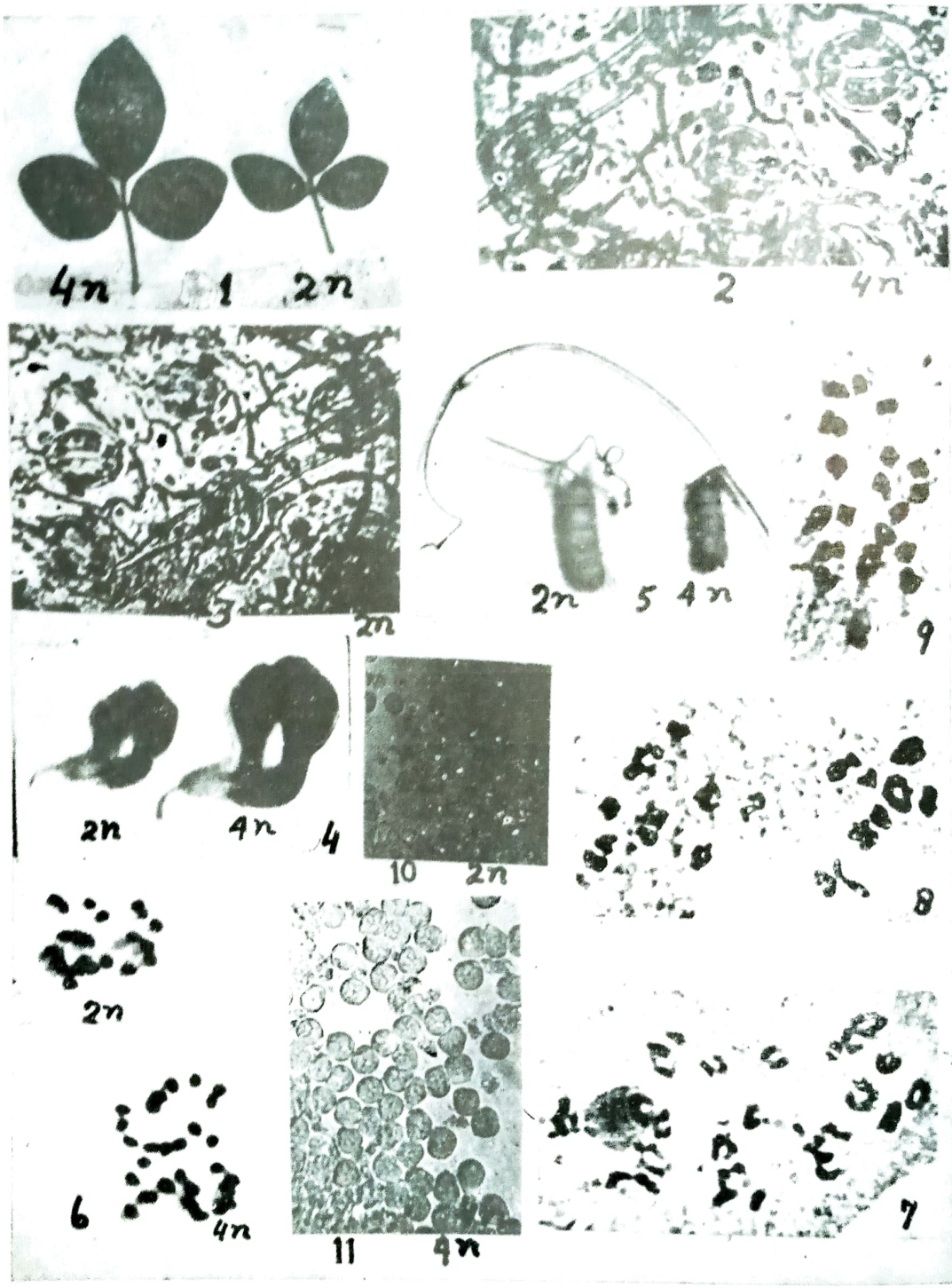
TABLE V

DISTRIBUTION OF CHROMOSOMES AT ANAPHASE I IN INDUCED TETRAPLOID

Distribution of chromosome	No. of PMCs	Percentage
22—0—22	39	78%
21—0—23	6	12%
20—0—24	3	6%
20—1—23	1	2%
20—2—22	1	2%

only while its diploid indicates it to be 98.2%. Randolph (1941) concluded that sterility in autotetraploid maize is largely controlled by specific genes or gene combination and it is chiefly physiological in nature. On the basis of findings discussed above it can be concluded safely that the principal causes of sterility in autotetraploids as well as in some allopolylploids are a series of disharmonies produced at various stages of sexual cycle of which the disturbance in the spindle and other external features of meiosis are among the most important.

So far as induced tetraploidy in *A. scarabaeoides* is concerned, it has, however, not shown much promising result in the present investigation for its economic exploitation except for its luxuriant growth and gigas habit of vegetative parts. Hence



Figs. 1-11. Fig. 1. Diploid and tetraploid leaves × ⅔ of the natural size.
Fig. 2. Tetraploid stomata × 450.
Fig. 3. Diploid stomata × 450.
Fig. 4. Diploid and tetraploid flowers × ⅔ of the natural size.
Fig. 5. Diploid and tetraploid fruits × ⅔ of the natural size.
Fig. 6. Diploid and tetraploid seeds × ½ of the natural size.
Fig. 7. A PMC at diakinesis showing 2 quadrivalents and × 18 bivalents × 1250.
Fig. 8. A PMC at metaphase I showing three quadrivalents, one trivalent, fourteen bivalents and one univalent × 1250.
Fig. 9. A PMC at metaphase I showing 22 bivalents × 1250.
Fig. 10. Diploid pollen grains × 450.
Fig. 11. Tetraploid pollen grains × 450.

tetraploidy in plants where reproductive parts are economically used have comparatively poor chance of competiting with diploids. Thus polyploid breeding is not very promising in such cases except where vegetative parts are used for fodder etc. From this point of view, Mehta and Swaminathan (1955) raised tetraploid barseem which proved far superior to diploid counterparts in forage value. But the aforesaid account does not underrate the prospect of polyploid breeding. The fact that chromosome pairing is under genetic control has a bright future in-chromosome engineering. Chromosome or sequence of chromosomes can be identified which control normal pairing and subsequent meiotic stages. Such chromosomes can be added to make the species more efficient in reproductive behaviour. Hence stable aneuploids or tetrasomics with specific chromosomes can be established in crop plants.

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