Development and characterization of tetraploid castor

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An attempt was made to induce polyploidy in castor using colchicine. Seeds of three castor genotypes *viz*., 48-1, DCS107 and AP41 were treated with colchicine at four different concentrations (1%, 0.5%, 0.3% & 0.1%). The seeds were soaked at three different durations (48h, 24h & 12h). A total of 600 treated plants from each genotype was sown in the field along with untreated control. Colchicine treatment at high concentration coupled with long duration impacted the germination. Nil or very low germination was noted in the treatment of 48h at 1% concentration. Germination percentage increased with reduced concentration and duration (Table 1).

Table 1. Effect of colchicine treatment on germination of seeds

Genotype	Treatment Concentration	Treatment Duration	Per cent germination
AP41	1%	48h	0
	0.5%	48h	16
	0.3%	48h	90
	0.1%	48h	94
	Control	48h	96
48-1	1%	48h	8
	0.5%	48h	34
	0.3%	48h	44
	0.1%	48h	84
	Control	48h	96
DCS107	1%	48h	10
	0.5%	48h	64
	0.3%	48h	58
	0.1%	48h	68
	Control	48h	80
AP41	1%	24h	10
	0.5%	24h	26
	0.3%	24h	30
	0.1%	24h	90
	Control	24h	76
48-1	1%	24h	16
	0.5%	24h	44
	0.3%	24h	70

	0.1%	24h	74
	Control	24h	70
DCS107	1%	24h	42
	0.5%	24h	58
	0.3%	24h	58
	0.1%	24h	56
	Control	24h	62
AP41	1%	12h	92
	0.5%	12h	86
	0.3%	12h	84
	0.1%	12h	92
	Control	12h	96
48-1	1%	12h	56
	0.5%	12h	60
	0.3%	12h	72
	0.1%	12h	84
	Control	12h	70
DCS107	1%	12h	68
	0.5%	12h	56
	0.3%	12h	78
	0.1%	12h	66
	Control	12h	82

The LD50 value for colchicine treatment was calculated based on Probit analysis using the survival percentage of the seedlings. As per the Probit curve, the LD50 was found to be 0.25% (2.44mg/l), 0.32% (3.21mg/l), and 0.33% (3.28mg/l) for 48h, 24h and 12h of treatment, respectively (Fig 1a, 1b, 1c). The highest rates of tetraploids were obtained in 0.33% colchicine treatment for 12 hours.

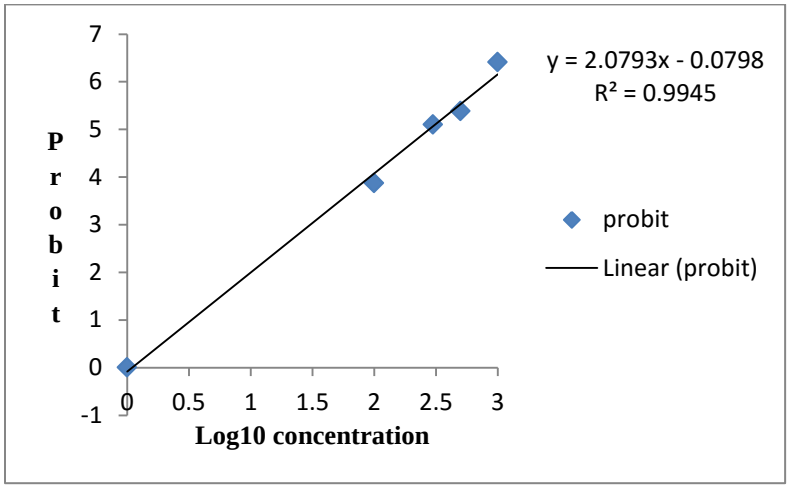


Fig 1a. Probit curve for calculating the LD50 value of colchicine treatment for castor: 48h

LD50=2.44 (mg/l)
0.24%

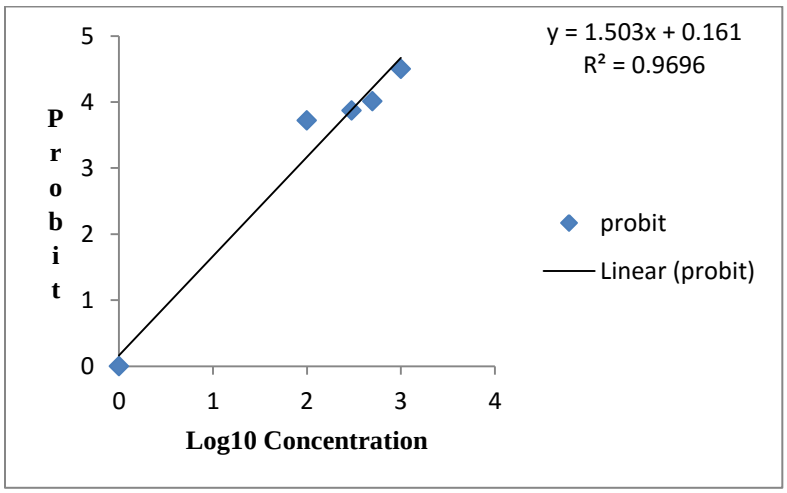


Fig 1a. Probit curve for calculating the LD50 value of colchicine treatment for castor: 24h

LD50=3.21 (mg/l)
0.32%

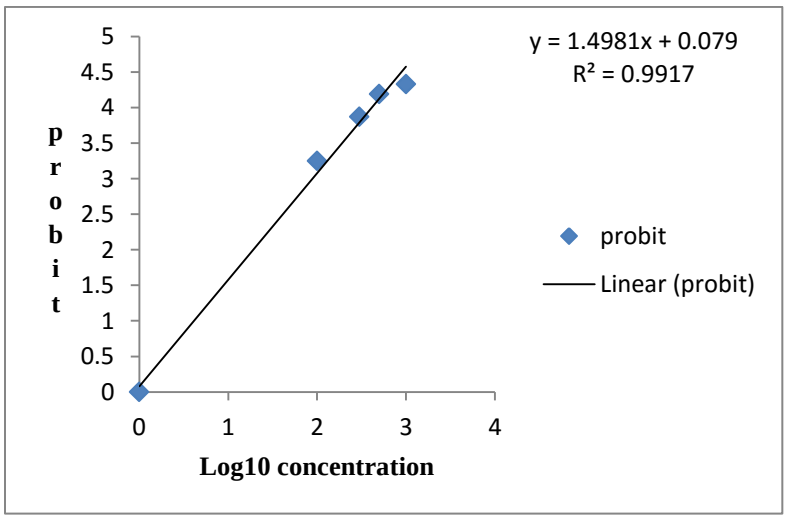
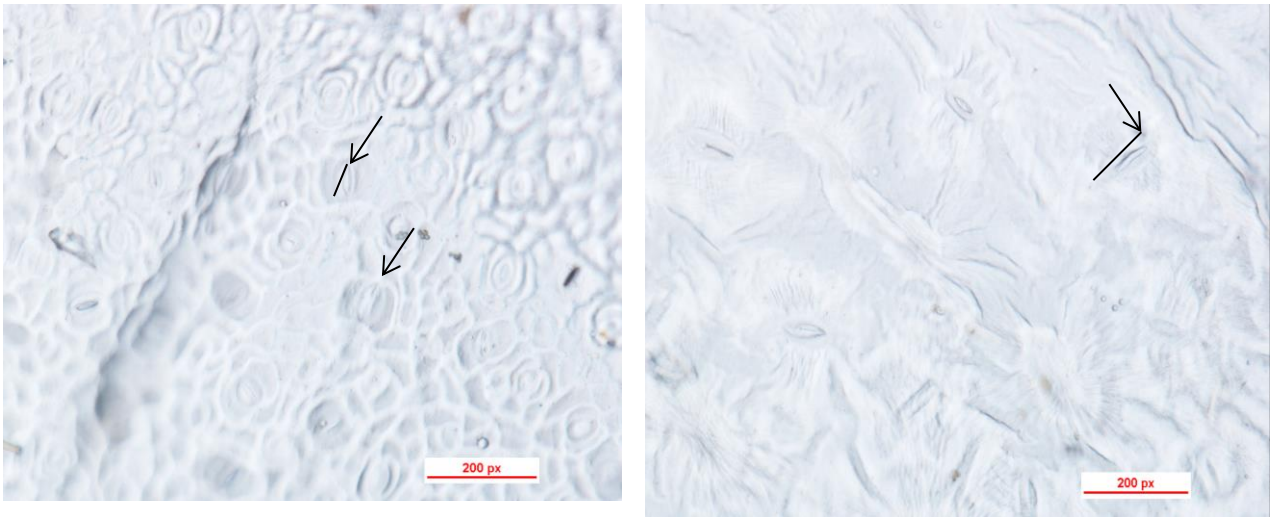


Fig 1a. Probit curve for calculating the LD50 value of colchicine treatment for castor: 12h

LD50=3.28 (mg/l)
0.32%

Initial screening of plants for polyploidy was done based on the evaluation of stomatal traits. The plants were screened for increase in the stomatal size and decrease in stomatal density in comparison with control. Three plants (two plants of 48-1 and one plant of AP41) with less number of stomata per unit area and increased stomatal size was found out of 1,800 treated plant evaluated (Fig 2). These plants were suspected to possess increased number of chromosomes.



Control plant Treated plant

Fig 2. Number and size of stomata in control and treated plants

Out of three plants, one plant (48-1) died subsequently. The pollen fertility of remaining two plants (one each from 48-1 and AP41) were severely affected. The pollen fertility was 0 per cent in the mutant of 48-1 and 15-20 per cent in mutant of AP41 during October and November 2014. However, the pollen fertility was observed to increase (20-25% in 48-1 and 30-35% in AP41) during the months of February and March 2015. Both the plants were subsequently selfed. Reduced seed setting with one or two cocci in a capsule containing aborted ovules was observed. About 15 seeds from each plant were harvested and sown in the field. A total of 12 progenies of 48-1 and 10 progenies of AP41 could be maintained. These plants were analyzed for pollen fertility and ploidy status through standard cytological procedures. The pollen fertility of progenies was found to vary from 3.89 to 54.72% in 48-1 and 32.46 to 62.32% in AP41 (Table 2).

Table 2. Fertility percent of the plants

Line	Plant No.	Fertility percentage
48-1	P1	19.39
48-1	P2	22.26
48-1	P3	9.42
48-1	P4	12.35
48-1	P5	20.4
48-1	P6	3.89
48-1	P7	9.25
48-1	P8	24.26
48-1	P9	23.52
48-1	P10	15.24
48-1	P11	26.22
48-1	P12	54.72
AP41	P1	32.46
AP41	P2	49.75
AP41	P3	37.93
AP41	P4	44.47
AP41	P5	57.2
AP41	P6	61.51
AP41	P7	48.34
AP41	P8	47.21
AP41	P9	49.8
AP41	P10	62.32

Out of 22 plants, seven highly sterile plants (4 of 48-1 and 3 of AP41) were subjected to meiotic analysis to count the chromosomes and study the pairing pattern. Male flower fixed from 7AM to 9AM were found to undergo sporogenesis. The fixation of flower based on male flower size varied for each genotype. Each male flower had anthers with different development stage therefore all the stages could be observed in a single male flower. The outer anthers matured earlier when compared to the inner ones. The staining with 1% acetocarmine did not work well in castor. Hence, 1% propionic carmine was used to view the pollen mother cells.

The PMCs with good spread, which were in different stages such as diakinesis, metaphase, anaphase and tetrad, were studied for chromosome association and movements. The chromosome associations were counted at diakinesis and chromosome behavior was studied at anaphase-I and in tetrad. At least 100 well spread cells were counted and the mean was calculated.

The control (diploid) plants showed ten bivalents in diakinesis and the immature pollen grains were observed to have one nuclei. The nucleolus organizer region (NOR) was found in two chromosomes. Pollen fertility was 99% and the pollen grains were of uniform size (Fig 3).

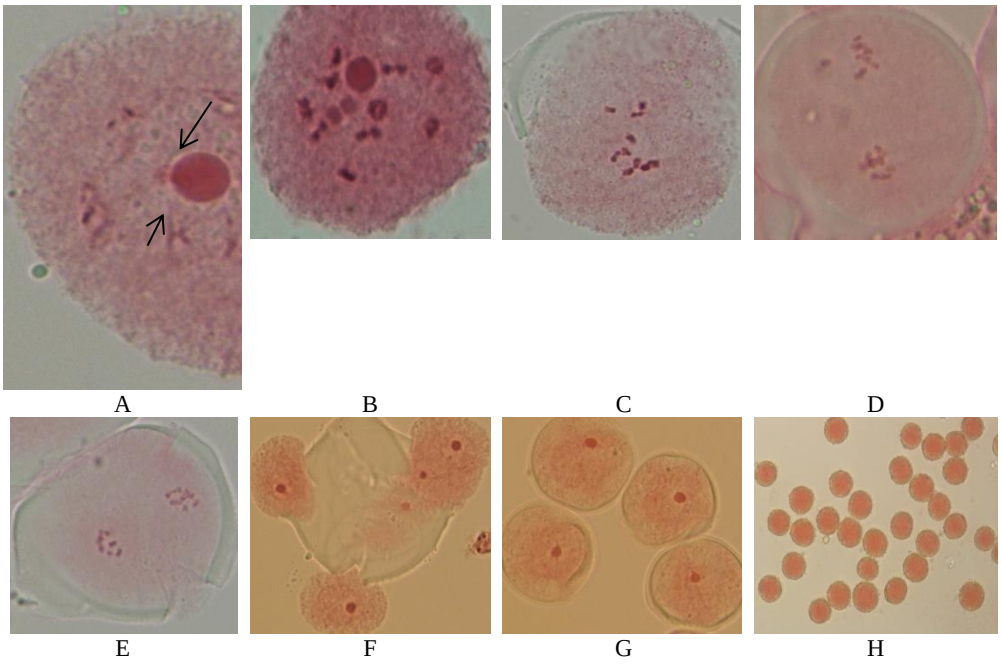


Fig 3. Cytological behavior of control (diploid) plant

A. Diplotene with two NOR (arrows indicating NOR)
B. Diakinesis with 10-II
C. Metaphase (10II),
D. Anaphase-I
E. Metaphase-II
F. & G. Microspores with single nucleus
H. Pollen fertility

The chromosome number in all selected progenies of colchicine treated plants were found to be doubled (2n=4x=40). The chromosome association in Diakinesis and Metaphase-I indicated tetraploid behavior with univalents, bivalents, quadrivalents and other associations (Table 3). The pairing was abnormal with higher chromosome configurations (Fig 4a to 4g) but the division was normal. Quadrivalent association was more frequently observed. Two chromosomes association with varied from four to six. The pollen fertility increases with increase in number of bivalent formation during Metaphase-I. Normal tetrad formation was observed. The immature pollen cells contained more than one nuclei. The pollen fertility of the plants varied from 3 to 60%. The plants of 48-1 recorded less fertility compared with AP41.

Table 3. Meiotic chromosome association at Diakinesis/ Metaphase-I, Sporad formation at the end of meiosis II and pollen fertility in different plants

Plant	Chromosome association at diakinesis / metaphase-I							Most frequent association	Number of nuclei	Pollen fertility (%)
	I	II	III	IV	V	VI	VIII			
48-1-P1	0-1	1-12	0-4	1-8	0-3	0-3	-	8II+6IV	1-5	19.39
48-1-P5	-	2-10	0-3	3-7	0-2	0-1	0-1	5II+6IV+1VI	1-3	20.40
48-1-P6	-	2-9	-	4-8	-	0-1	0-1	9II+4IV+1VI	2-4	3.89
48-1-P10	0-1	2-11	0-2	0-9	-	0-1	-	4II+8IV	1-2	15.24
AP41-P1	-	6-16	0	2-7	-	-	-	12II+4IV	1-3	32.46
AP41-P2	0-1	5-14	0-1	4-6	-	0-1	-	14II+3IV	1-2	49.75
AP41-P10	-	6-16	-	2-7	-	-	-	16II+2IV	1-2	62.32

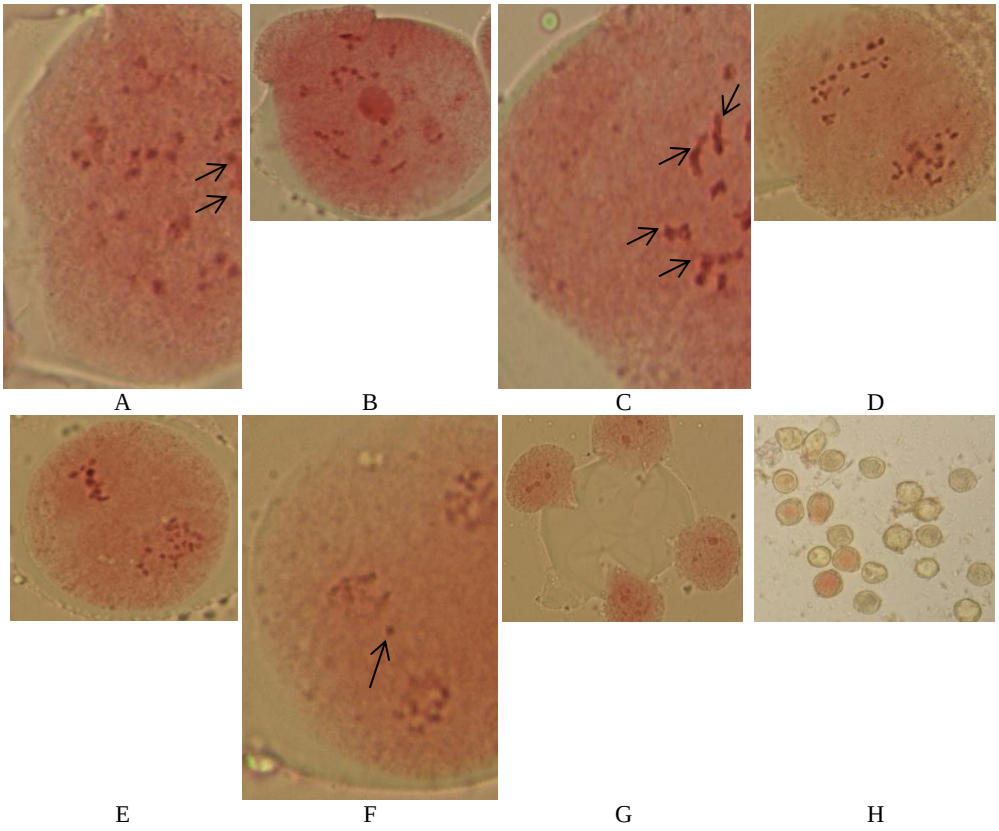


Fig 4a. Cytological behavior of 48-1-1
A. Four NOR association (arrows indicating NOR),
B. Diakinesis with 1I+12II+3III+1IV+1V
C. Metaphase with 8II + 6 IV (arrows indicating quadrivalent)
D. Anaphase-I
E. Metaphase-II (Equatorial and Polar view
F. Anaphase-II with laggards
G. Microspore with more than two nuclei
H. Pollen fertility with unviable pollen

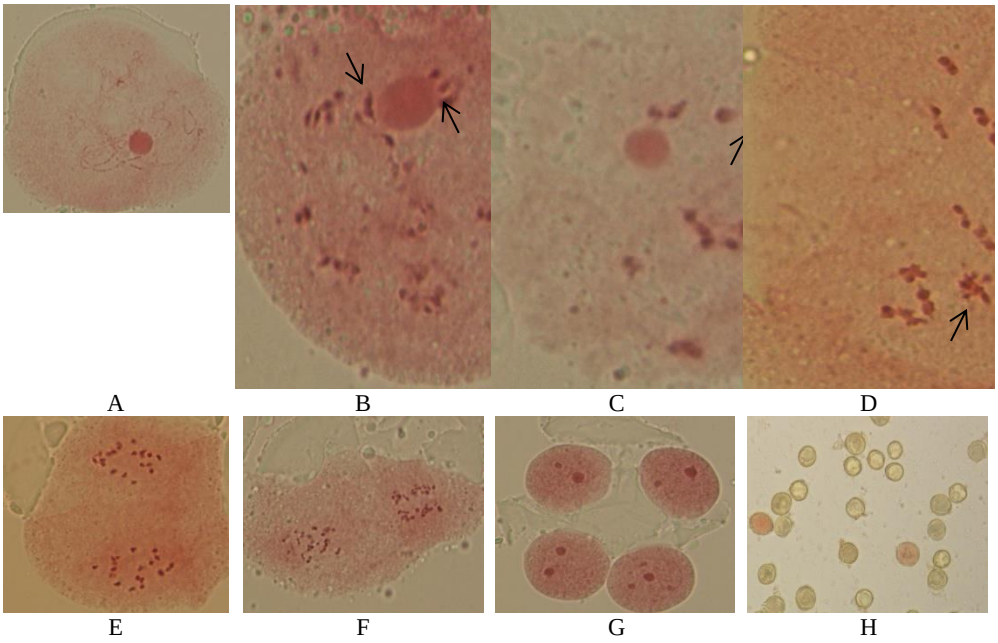


Fig 4b. Cytological behavior of 48-1-5

A. Pachytene
B. Four NOR association (arrows indicating NOR)
C. Diakinesis 5II+6IV+1VI
D. Metaphase 5II+6IV+1VI (arrows indicating hexavalent)
E. Anaphase-I
F. Metaphase-II & early anaphase-II
G. Microspore with more than two nuclei
H. Pollen fertility with unviable pollen

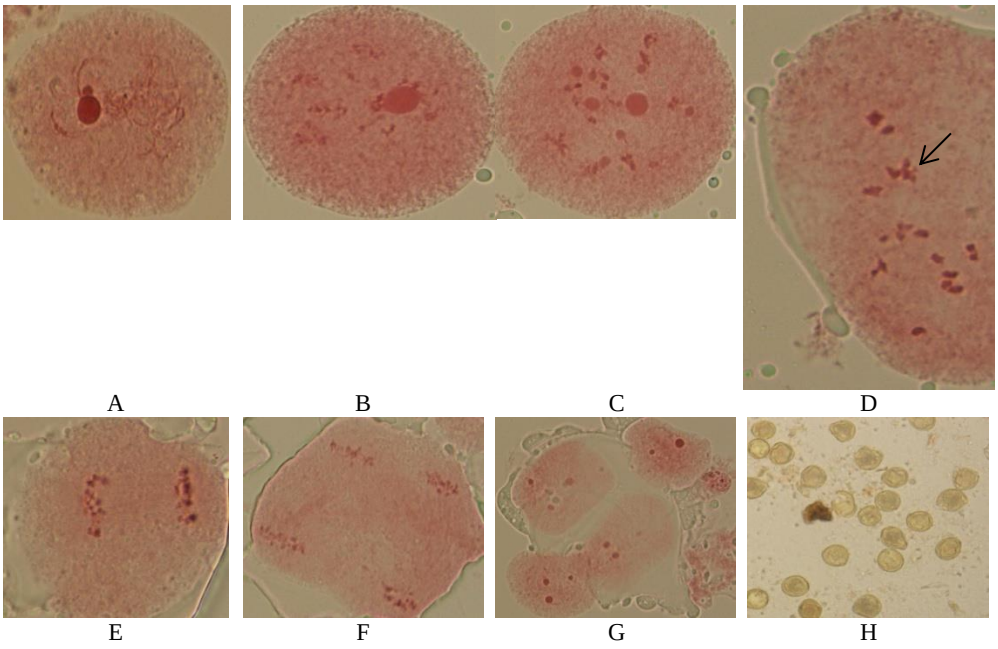


Fig 4c. Cytological behavior of 48-1-6

A. Pachytene
B. Four NOR association
C. Diakinasis
D. Metaphase-I 9II+4IV+1VI (arrows indicating hexavalent)
E. Anaphase-I
F. Anaphase-II
G. Microspore with more than two nuclei
H. Sterile pollen

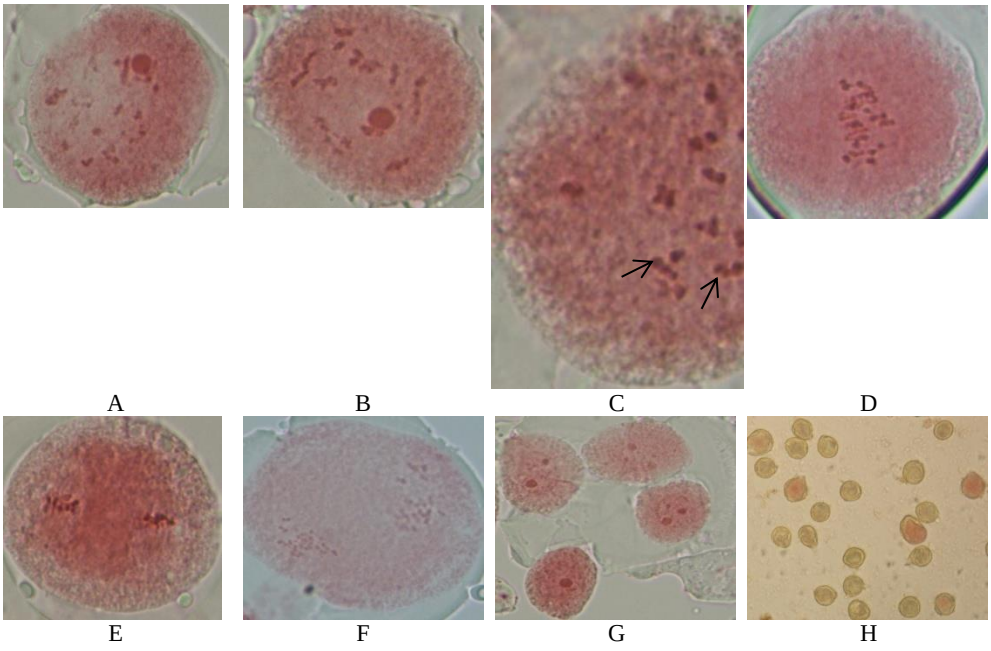


Fig 4d. Cytological behavior of 48-1-10
A. Four NOR association
B. Diakinesis 4I+6II+6IV
C. Metaphase-I 11II+2III+3IV (arrows indicating quadrivalent)
D. Early Anaphase-I
E. Metaphase-II
F. Anaphase-II
G. Microspore with more than two nuclei
H. Sterile pollen

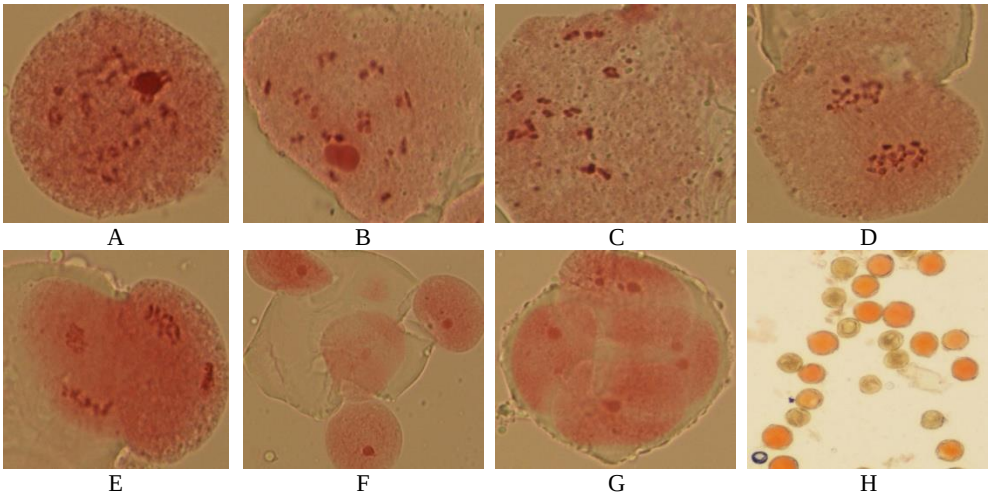


Fig 4e. Cytological behavior of AP41-13
A. Four NOR association
B. Diakinesis 16II+2IV
C. Metaphase 12II+4IV
D. Anaphase-I
E. Anaphase-II
F. & G. Microspore with more than two nuclei
H. Fertile and Sterile pollen

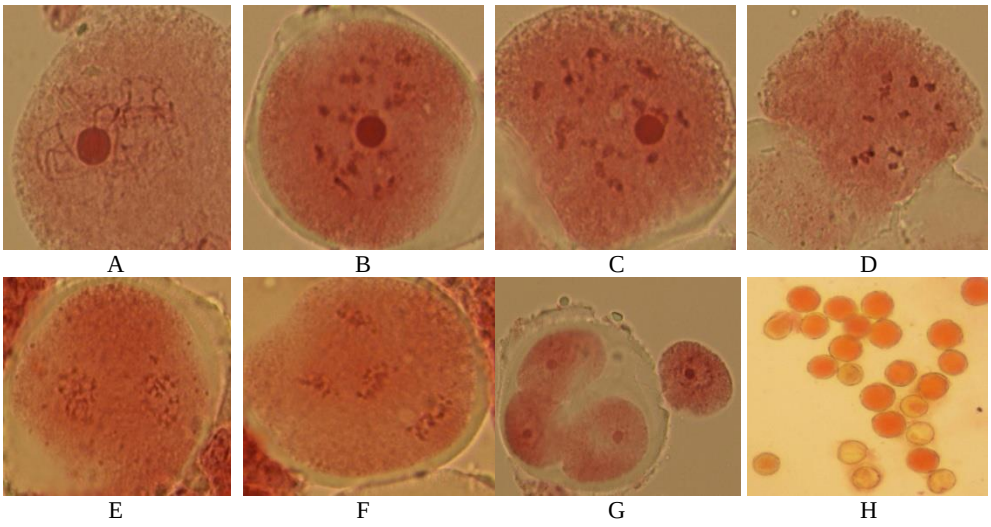


Fig 4f. Cytological behavior of AP41-14

A. Pachytene
B. Four NOR association
C. Diakinasis 14II+3IV
D. Metaphase-I 14II+3IV
E. Anaphase-I
F. Anaphase-II
G. Microspore
H. Pollen fertility

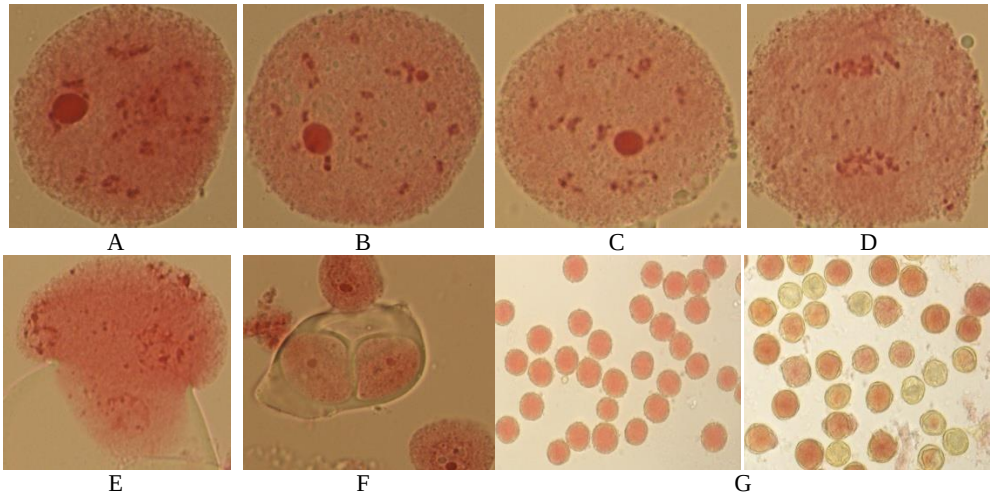


Fig 4g. Cytological behavior of AP41-22

A. Four NOR association
B. Diakinesis 16II+2IV
C. Diakinesis 14II+3IV
D. Anaphase-I
E. Anaphase-II
F. Microspore
G. Pollen fertility