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Meiotic Behaviour and Pollen Fertility in Colchiploid *Zea mays* L.

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Abstract
Colchicine at various concentration was given to the apical buds of maize (*Zea mays* L.). Plants, in order to induce tetraploidy for meiotic studies. Highly irregular meiosis in the C_1 generation was revealed. These meiotic abnormalities increased pollen sterility which lead to a lower seed setting. In the C_2 generation the occurrence of bivalents has increased and that of multivalents has decreased. Restoration of meiotic regularities, pollen fertility.

Introduction
Disturbances at premeiotic association have been identified by Driscoll *et al*. (1967), Bennett and Smith (1979), Puertar *et al*. (1984), Hassan and Jones (1994, 1995) and Loidl (1988, 1989). Colchicine treatment applied to the apical buds of the plants induces tetraploidy. Induced polyloidy is known to enhance the production of plants where vegetative organs and their biomass constitute the economic product (Lavania, 1986). It is well known that experimentally produced autotetraploids show a reduced fertility as related to their diploid counterparts. This is true for the pollen as well as the seed fertility. With regard to the seed fertility, the situation is considerably worse. In the family Polemoniaceae, genus Phlox (2n = 14) has mainly horticultural values in which ploidy was induced to increase flower size as well as seed out put, with a hope that enough variability could be created by the induction of polyploidy and selection from segregation generation which would yield better genotype for the improvement of the plant. In the present study chromosome pairing behaviour and pollen fertility in maize were investigated up to second generation (C_2).

Materials and Methods
The seeds of *Zea mays* were collected from the healthy plants at the experimental farm of the Department of Genetics & Plant Breeding, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh. Seeds were tested for their diploid character and normal feature of meiosis. The colchicine solution was applied to the apical buds of the plant (Singh and Roy, 1984).

First generation (C_1): The effects induced by colchicine were measured by following parameters.

Meiotic abnormalities: For meiotice studies, flower buds were fixed in acetic-alcohol (1:3) with a few drop of ferric chloride in it to facilitate staining. Squashes and slide preparation were made in the usual way (Hassan and Jones, 1995).

Pollen fertility: Heating the pollen grains in a drop of 1 per cent aceto-carmine solution, those which suitably stained were observed as fertile and the empty, shrunken ones as sterile. Fertility percentage was scored on the average of 100 pollen grains.

Second generation (C_2): The seeds were collected from C_1 generation and sown for the study of C_2 generation.

Meiotic abnormalities: Second generation meiocytes were examined on the lines of first generation (C_1).

Results and Discussion
Observation on induced tetraploid in maize was extended over first (C_1) and second (C_2) generation using meiotic abnormalities and pollen fertility. Colchicine solution (0.20 to 0.50 per cent concentration) applied to the apical bud of which 0.25 and 0.35 per cent concentration inducing tetraploidy in which 0.25 per cent concentration was found more effective (Table 1). Seeds were collected from C_1 generation by selfing and sown for the study of C_2 generation which were also found tetraploid on being cytologically investigated but their frequency was low.

Table 1: Colchicine treatment on seedling of *Zea mays* (days = 3; hour = 8; n = 25)

Conc.%	Induction of tetraploidy	Percentage of tetraploidy
0.20	-	-
0.25	-	36
0.35	3	12
0.40	-	-
0.50	-	-

Meiotic abnormalities: The chromosome configuration of the induced colchiploid maize showed high degree of meiotic irregularities in C_1 generation. Meiosis in diploid showed 10 bivalents at diakinesis with regular divisions and cytokinesis led to the formation of normal tetrads while colchicine induced autoploids revealed x = 20 chromosomes at diakinesis and metaphase I. In the treated plants univalents, bivalents and multivalents appeared with inconsistent frequencies in C_1 and C_2 generation. The chromosomal association in C_1 generation were variable with and average of 1.86 univalents, 16.56 bivalents and 1.31 tetravalents. There was total absence of trivalents at diakinesis while few PMCs showed all bivalents and few has bivalents and univalents. In C_1 generation, the chromosome configuration per PMC was 1.00 univalents and 18.90 bivalents. There was a decrease or total absence of multivalents and an increase in bivalents which improve to chiasma frequency per nucleus or bivalents. Chiasma frequency was directly related to the chromosomal configuration and due to an increase in the number of chromosomes per cell in the autotetraploid, the chiasma frequency increased but in this experiment decreased from 1.64 chiasma per bivalent (18.50 chiasma/nucleus) in diploid to 1.30 chiasma per bivalent in autoteraploid (33.50 chiasma/nucleus) and recovered in C_2 generation i.e. 1.69 chiasma per bivalent (23.35 chiasma/nucleus) (Table 2).

The irregular chromosome behaviour resulted into maximum meiotic abnormalities in C_1 generation were less in C_2 generation. Abnormalities such as stikiness, laggards, bridges and uniqual separation of chromosomes were more at anaphase I and II in C_1 than C_2 generation. In C_1 generation, laggards varied from 4 to 6 and ascribed to the anomaly of spindle or acentric fragment. It causes irregular distribution of chromosomes at poles and fail to reach the poles and discarded. Unequal distribution of chromosomes lead to the formation of chromosomally imbalanced daughter nuclei in C_1 generation. The lesser abnormalities in C_2 generation was due to the presence of normal separation of chromosomes (Table 3).

Pollen fertility: Pollen strility was the direct result of induced abnormalities which increased due to cytological abnormalities. Pollen sterility was 6.66 per cent in diploid, 23.48 per cent in C_1 generation and 15.81 per cent in C_2 generation. There was a significant recovery from C_1 to C_2 generation of the plant caused by genetic damage.

Colchicine has mutagenic properties and there is also a wealth of literature concerning its physiological effects on both plants animal tissued. Colchicine can affect characters associated with chromosome pairing behaviour at meiosis. The effect of induced polyploidy is an increase in cell size as was apparent in induced tetraploid from the size of guard cells and pollen grains and also reported in Delt cardinale (Mehlquist *et al*., 1943), in Delphinium (Singh and Roy, 1983), Antirrhinum (Mahal *et al*., 1968) and Balsam (Jalil *et al*., 1974). These increase seem to be pleiotropic resulting into an increase in almost all the determinate organs: seed, floral parts, leaf size and thickness, stem thickness and height. The induced autoploids continued to show characters which made them superior and late in maturity. A change in petal intesity was yet another useful variation produced by the diploidization of genome. Above these features makes the tetraploid flowers move attractive (Singh and Roy, 1981).

Meiotic analysis of diploid revelaed all bivalents at diakinesis as well as metaphase I, while meiotic instability of autoteraploids increases with increasing level of ploidy. An average chromosome confiuration of tetraploid maize was 2.01I+ 16.89II+ 1.061IV against 10II in control at diakinesis. The frequency of tetravalents in C_1 generation ranged from 0-2 per cell while in C_2 generation the tetravalents were generally absent. The meiocytes of C_2 generation showed 0.78+19.30II per PMC. It is generally assumed that, the number of quadrivalents is governed by the size of the chromosomes, chiasma frequency and excess of chromosomes within the cell which interfare with normal pairing. The less number of quadrivalents have been also reported in sunflower (Dhesi and Saini, 1973), Verbnea (Arora, 1940), Portulaca (Singh, 1979), *Helianthus annuus* (Gupta and Roy, 1986), Petunia hybrida (Singh and Roy, 1988), Solanum Khasianum (Bhatt, 1977), Gossypium (Beasley, 1940).

The chiasma initiation at telomeric sites are possible and interdependence of chiasmata in a bivalent (as evident from dominance of rod bivalents in autotetraploids) reduces the chances of quadrivalent formation in tetraploids leading to cytogenetic diploidization of colchiploids (Lavania, 1986). There had been reduction in the chiasma frequency of the tetraploids; the mean frequency being 33.50 per cent and 1.30 per bivalent. In C_2 generation these increased to 23.35 per cell due to stabilization or reduce number of quadrivalents. The consequence of meiotic aberrations can be evaluated by recording pollen and plant sterility. The detail cytological observation, made during present study have sown the occurrence of multiple associations which led to meiotic abnormalities and these irregularities are considered to be due to the formation of multivalent associations (Darlington, 1965). The cytological anomalies such as univalent formation, laggards, bridges, unequal distribution of chromosomes at anaphase were observed in present autoploid plant. The univalent formation could be due to the failure of pairing, non-disjunction of high homogeneity occurring among the chromosomes and lagging to the failure of moving apart. The bridges could be due to inversion or dicentrics but how these could have arisen with polyploidy, cannot be explained. With reference to above meiotic abnormalities discussed, it is highly probable that irregularities such as irregular distribution of univalents and laggards at anaphase I and univalents of laggards which fail to reach the pole at telophase I may lead to the formation of micronuclei. The number of micronuclei were very common in the first generation while their number is reduced in subsequent generation. The frequency of quadrivalents in autotetraploid varies from cell to cell and from species to species.

There is direct relationship between chiasmata and polled fertility, multivalents reducing the latter, which is evident from observations that C_2 generation of plants have less

Table 2: Chromosome association and chiasma frequency in diploid and autotetraploid *Zea mays*

Generation	PMCs studied		Mean frequency of nucleus Univ.	Bi.	Tri.	Tetra.	Chiasma/ nucleus	Chiasma/ bivalent	Pollen sterility
Diploid	50	Mean	-	10.00	-	-	18.50	1.64	9.92
2n= 2x = 20)		range	-	(10)	-		(18-19)		
Autotetraploid									
(2n=4x = 40)									
C1	50	Mean	1.86	16.56	-	1.31	33.50	1.30	26.99
		range	(0-6)	(12-18)	-	(0.2)	30-35)		
C2	50	Mean	1.00	18.90	-	-	23.35	1.59	16.26
		range	(0-4)	16-19)	-	-	(23-25)		

Table 3: Chromosome behaviour at anaphase and telophase

Generation	PMCs studied	Anaphase I/Telophase I Laggards	Bridges	Laggards + bridges	Anaphase II/Telophase II Laggards	Bridges	Laggards + bridges	Spored stage 5 daughter nuclei	Poly spored	Total abn. PMCs
C1	100	12.56	3.22	1.09	9.88	1.40	1.50	7.88	-	48.23
C2	100	6.96	0.88	-	5.33	0.42	-	4.44	-	20.11

number of tetravelents and high pollen fertility than those of C_2 generation. Hazarika and Rees (1967) reported that univalents and multivalents due to segregational errors lower the fertility in autotraploids. Dominance of bivalents imparted fertility in autotetraploid sunflower and petunia hybrida (Gupta and Roy, 1979; Singh and Roy, 1980). Such autotetraploid plants with preponderance of bivalents have been recorded by other workers (Forbes and Burton, 1963; Harlan and de Wet, 1969). Kostoff (1940) confirmed that remarkable recovery of pollen fertility in subsequent generation due to the chromosome association tend change to normal pattern having lesser number of uni- and tetravaents. Pollen sterility in autotetraploid *Zea mays* caused less fruit and seed formation. Lower seed production has been the chief factor for excluding autopolyploidy as a regular breeding method for improvement of the crop. In the present study, autotetraploid plants consistently produced lesser fruits irresapective of generation. Healthy seeds were more in C_2 generation. The seed fertility in the colchiploid is required for improved propagation because of the reduction in quadrivalent frequency and subsequent balanced anaphase separation and ultimately the induced plants improved the quality as a whole.

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