J. Indian bot. Soc. **65** : 141—149, 1986

# STUDIES IN COLCHICINE-INDUCED TETRAPLOID OF *PENNISETUM TYPHOIDES* STAPF *ET* HUBB[1]

R. P. JHA AND R. R. P. SINHA

*Botany Department, Patna University, Patna*

## ABSTRACT

Induced tetraploids in *P. typhoides* were obtained by treatment with 0.25% aqueous colchicine solution. Their morphological and cytological studies were made in comparison to diploid plants of this species. Induced tetraploids showed thicker stem with shorter internodes, deeper green and larger leaves having larger stomata, bigger mesophyll cells, pollen grains, spikelets etc, but plant height, number of tillers per plant, yield of seeds, number of stomata per unit surface area etc. decreased and flowering was delayed in them. They showed somatic chromosomes in groups of four and several meiotic irregularities such as formation of univalents, multivalents, unequal separation of chromosomes at anaphase I, bridges, lagging chromosomes, micronuclei etc. Seed setting was very poor in them.

## INTRODUCTION

Polyploidy has been induced in many forage grasses. Mehta and Swaminathan (1955, 1977) induced polyploidy in *Trifolium alexandrium* and other forage crops, Ahloowalia (1967) in rye grass, Magoon and Tayyab (1968) in *Sorghum* species, and many other workers in many more species of grasses. Jauhar (1970) raised synthetic tetraploids in *Pennisetum typhoides*, but polylploid effect receded in subsequent generations leading toward diploidisation. Hence his studies could not be of great economic importance. But they may be important for raising triploids by crossing with diploids and triploid may have some economic importance. Triploid sugarbeets yielded more sugar than diploid (Peto and Boyes, 1940). The present work has been taken up by authors in *P. typhoides* with this aim of improvement of crops and the present paper which deals only with production of colechitetraploids forms the first step in this programmes.

## MATERIALS AND METHODS

(i) *Seed treatment*—Healthy seeds of *Pennisetum typhoides* var. T-55 were immersed in 0.1%, 0.2% 0.25%, 0.4% and 0.5% colchicine solutions for 4 to 8 hours, washed thoroughly with water and sown in well manured soil. (ii) *Seedling treatment*—Growing tips of one week old seedlings were immersed in above colchicine solutions in capillary tubes for 4 to 12 hours. Morphological and cytological studies were made in treated plants (mitotic studies from root tip squash and meiotic studies from young anther squash). Root tips were pretreated with saturated aqueous solution of alpha-bromonaphthalane for 30 to 45 minutes at room temperature. Materials were fixed for 48 hours at low temperature in acetic alcohol

1 Accepted for publication on February 19, 1985.

The authors are grateful to Professor R. P. Roy, former Head of the Department of Botany, Patna University for his guidance and encouragement in course of the present investigations.

(1 : 3) with a few drops of ferric chloride (mordant) and stained in acetocarmine.

## OBSERVATIONS

The mortality rate was very high in colchicine treated seedlings. Out of 200 seedlings 20 survived to matuity and only 4 turned out to be tetraploid by treatment with 0.25% colchicine solution. The immediate effect of colchicine treatment was inhibition of growth and swelling of coleoptile just beneath apical region. The coleoptile formed callus like thickenings. The effect of colchicine treatment was determined by making morphological and cytological investigations.

*Morphological studies*—Comparative studies of morphological characters in diploid and colchitetraploid plants have been detailed in the following table :

TABLE I

MORPHOLOGICAL CHARACTERS OF DIPLOIDS AND INDUCED TETRAPLOIDS

| Characters | Diploid | Tetraploid |
|---|---|---|
| Plant height (cm) | 166.30±4.32 | 152.00±4.54 |
| No. of tillers | 4.00±0.08 | 3.50±0.07 |
| Diameter of stem (cm) | 1.50±0.08 | 1.90±0.12 |
| Length of Leaf blade (cm) | 48.00±2.42 | 56.00±3.49 |
| Width of leaf blade (cm) | 3.20±0.11 | 3.80±0.14 |
| Surface of leaf blade | Somewhat coriaceous, very sparsely hairy. | Coriaceous, hairy. |
| Colour of leaf blade | Light green | Dark green |
| Length of spike (cm) | 15.50±0.70 | 16.20±0.74 |
| Diameter of spike (cm) | 2.50±0.09 | 3.00±0.11 |
| Length of spikelet (mm) | 5.00±0.15 | 6.20±0.17 |
| Length of anther (mm) | 2.20±0.09 | 3.00±0.11 |
| No. of stomata per field (10×15) on lower epidermis | 13.50±0.35 | 9.80±0.38 |
| Length of guard cells (μm) | 20.30±0.30 | 26.60±0.38 |
| Breadth of guard cells (μm) | 9.00±0.20 | 12.50±0.25 |
| Length of stomatal aperture (μm) | 12.20±0.22 | 16.50±0.29 |
| Breadth of stomatal aperture (μm) | 4.50±0.10 | 5.80±0.12 |
| Diameter of pollen grains (μm) | 32.30±0.46 | 46.20±1.82 |
| Pollen fertility (%) | 96.00 | 76.00 |
| Seeds set per earhead | 450.00±18.00 | 142.00±6.00 |
| Weight of 100 seeds (gm) | 0.56±0.02 | 0.89±0.02 |
| Days to flower | 75.00±2.50 | 91.00±3.10 |

*Cytologiacl studies* : *Mitosis*—In diploid plants 2n=14 and in induced tetraploid plants 2n=28 (Figs. 7 and 8). Somatic chromosomes are in groups of four in treated 4n plants and their total chromatin length is 86.96$\mu$ and T. F. % is 44.84 (42.74$\mu$ and 45.01 respectively in 2n plants).

*Meiosis*—Diploid plants showed 7 bivalents at metaphase I and normal eparation of chromosomes at anaphase I. nduced tetraploids, on the other hand, howed univalents, trivalents and quadrivalents besides bivalents at metaphase Figs. 9 and 10). One quadrivalent or wo bivalents were invariably asssociated with nucleolus. The chiasm frequency and frequency of univalents bivalents, trivalents and quadrivalents are summarised in Tables II an' III.

Quadrivalent configurations are of ring, chain Y, frying pan and shovel-shaped types. But ring quadrivalents were very common.

In 4n PMCs, at anaphase I, normal separation of 14-14 chromosomes to both poles occurred in 15% PMCs while unequal separation was observed in 35% PMCs (Table IV). Univalents reached the poles either divided or undivided, or they lagged behind. 50% PMCs at anaphase I showed laggards, bridges and lagging bivalents (Tables IV and V).

Chromatin bridges with or without fragment were also observed (Fig. 11). In some PMCs certain bivalents did not readily separate at anaphase I. The configuration of such a belated separation of chromosomes was quite distinct from that of a bridge. Laggards were also observed at telophase I. Divided or undivided univalents which failed to be included in daughter nuclei formed micronuclei.

At metaphase II and also at anaphase II and telophase II, a few chromosomes remained scattered in the cytoplasm and could not be incorporated in daughter nuclei. In some PMCs more than 4 polar groups of chromosomes were observed. This resulted in the formation of pentads.

Pollen grains of induced tetraploids were bigger in size than those of diploids (Figs. 2 and 3). Pollen grains also showed variation in size and pollen fertility was reduced in 4n plants.

Seeds obtained from induced tetraploids by selfing were sown in the soil and plants of $C_2$ generation were also found to be tetraploid on being cytologically investigated. Their morphological and cytological behaviour was like tetraploid plants of $C_1$ generation.

## DISCUSSION

Two features of induced tetraploids need examination. The first is their differential performance in the morphological and reproductive behaviours and the second their economic potentiality in the crop breeding programme. The first aspect i.e. in what way the induced polyploids are superior or inferior to their diploid counterparts may first be discussed. It has been observed that in general tetraploid *Pennisetum typhoids* show robustness of gigas habit in morphological characters. In most of induced tetraploids this appears to be the trend as is evident from the literatures (Newcomer, 1941 ; Tandon, 1950, and 1955; Bali and Tandon, 1957, and 1959, Tandon and Bali, 1957; Celarier and Mehra, 1958 ; Jauhar, 1970 ; Roy's PL 480 report, 1972). At earlier stage slow rate of growth and less number of tillers have also been reported in large number ofinduced tetraploids (Magoon, *et al.*, 1958 ; Dyck and Rajhathy, 1965 and Ahlowalia, 1967). Several types of explanations such as lowering down of physiological activities, unbalanced nature of modifying

TABLE

CHROMOSOME PAIRING AND CHIASMA

| Name of the species | No. of PMCs analysed | No. of univalents per PMC | | No. of bivalents per PMC | | | | No. of trivalents per PMC | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Rod | | Ring | | | |
| | | Range | Mean | Range | Mean | Range | Mean | Range | Mean |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| *P. typhoides* (diploid) 2n=14 | 100 | 0—2 | 0.24 | 2—4 | 2.33 | 3.5 | 4.55 | — | — |
| *P. typhoides* (induced tetraploid) 2n =28 | 50 | 0—3 | 0.98 | 2—3 | 2.40 | 5—12 | 6.54 | 0—1 | 0.30 |

genes etc. have been adduced or advanced for delayed growth and poor tillering (Magoon, *et al.*, 1958). The poor reproductive performance of induced tetraploids has mostly been explained due to a number of cytological situations that are found in tetraploids and are absent from diploids. The various types of quadrivalent configurations and their irregular seggregation lead to unbalanced gametes resulting in poor fertility. Due to those cytological irregularities induced tetraploids were found to be inferior to diploids in *P.radiatus* (Kumar and Abraham. 1942) *P. mungo* (Sen and Cheddo, 1958), *Dolichos biflorus* (Sen and Vidyabhushan, 1960) *Soraghum vulgare* (Schertz, 1962), *Melilotus indica* (Mehta *et al.*, 1965), *Cyamopsis psoralioides* and *P. aconitifolius* (Mishra, 1968) and also in a number of cucurbits (Roy, 1972). Even in the case of *P. typhoides*, Jauhar(1970) reported very high frequency of multivalents in induced tetraploids. In the present investigations only 4% of PMCs had only bivalents but the rest of the PMCs showed a mixture of bivalents, trivalents, quadrivalents, univalents, etc. (Table III). It has been established wherever critical studies have been made, that the chromosome pairing is under genetic control. Perfect bivalent formation as occurs in sexual species is the opical stage in the evolutionary adaptiveness of the species. Even duplication of the 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 the two sets do. Moreover, the occurrence of asynaptic and desynaptic cases induced diploid from haploid *Pelargonium* (Daker, 1967 ; Riley and Chapman, 1958 and several others). It clearly indicates strict genetic control of chromosome pairing and its subsequent behaviour. Hence, it is not at all surprising that the induced tetraploid displays a series of cytological irregularities at meiosis leading ultimately to reproductive deficiency. There are also reports that the rare frequency of bivalents/multivalents in tetraploids shows differences in the different strains of the same species. Some strains at the tetraploid level have more of bivalents and less of multivalents whereas other strains may show the opposite effects. A situation of this type has been lucidly explained in *Sorghum nitens* and *sorghum nitidum* by Magoon and Tayyab, 1968. In the previous species mean quadrivalent

II

FREQUENCY AT METAPHASE I

| No. of quadrivalents per PMC | | Chiasmata per PMC | | | Terminalised Chiasmata per PMC | | | Chiasma per chromosome | Terminalisation co-efficient |
|---|---|---|---|---|---|---|---|---|---|
| Range | Mean | Range | Mean | S.E.± | Range | Mean | S.E. ± | | |
| 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| — | — | 9–12 | 11.50 | 0.22 | 9—12 | 11.30 | 0.21 | 0.821 | 0.982 |
| 0–4 | 2.06 | 18—24 | 21.20 | 0.27 | 18—24 | 20.60 | 0.35 | 0.757 | 0.971 |

TABLE III

FREQUENCY OF TYPES OF CHROMOSOME CONFIGURATIONS IN INDUCED TETRAPLOIDS

| No. of PMCs | Chromosome configurations | Percentage |
|---|---|---|
| 8 | $^{4}IV + {}^{0}III + {}^{6}II + {}^{0}I$ | 16 |
| 6 | $^{1}IV + {}^{0}III + {}^{12}II + {}^{0}I$ | 12 |
| 6 | $^{1}IV + {}^{0}III + {}^{11}II + {}^{2}I$ | 12 |
| 6 | $^{0}IV + {}^{1}III + {}^{12}II + {}^{1}I$ | 12 |
| 5 | $^{2}IV + {}^{0}III + {}^{10}II + {}^{0}I$ | 10 |
| 4 | $^{4}IV + {}^{1}III + {}^{3}II + {}^{3}I$ | 8 |
| 3 | $^{4}IV + {}^{1}III + {}^{4}II + {}^{1}I$ | 6 |
| 3 | $^{3}IV + {}^{0}III + {}^{8}II + {}^{0}I$ | 6 |
| 3 | $^{2}IV + {}^{0}III + {}^{9}II + {}^{2}I$ | 6 |
| 2 | $^{3}IV + {}^{1}III + {}^{6}II + {}^{3}I$ | 4 |
| 2 | $^{0}IV + {}^{0}III + {}^{13}II + {}^{3}I$ | 4 |
| 2 | $^{0}IV + {}^{0}III + {}^{14}II + {}^{0}I$ | 4 |
| Average per PMC | 2.06±0.42 | 0.3±0.16 8.94±087 0.98±0.26 |

TABLE IV

DISTRIBUTION OF CHROMOSOMES AT ANAPHASE I IN INDUCED TETRAPLOIDS

| No. of PMCs | Distribution | Percentage |
|---|---|---|
| 15 | 14—14 | 15 |
| 10 | 15—13 | 10 |
| 9 | 16—12 | 9 |
| 6 | 17—11 | 6 |
| 5 | 18—10 | 5 |
| 2 | 19— 9 | 2 |
| 3 | 20— 8 | 3 |
| 50 | Laggards, bridges & lagging bivalents | 50 |

TABLE V

FREQUENCY OF LAGGARDS, BRIDGES AND LAGGING BIVALENTS IN PMCS OF INDUCED TETRAPLOIDS

| Total no. of PMCs analysed 100 | Number of Lagging chromosomes | | | | | | | | Bridges | Lagging bivalents |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | | |
| No. of PMCs | 50 | 10 | 9 | 10 | 6 | 3 | 4 | 1 | 3 | 2 |

is less than 2/3rd whereas in the latter, it is more than 2/3rd. Probably in the former species a certain genetic control reduces quadrivalent frequencies and increases diploidisation. This type of genetic control is also reflected in the direction of chiasma frequencies in the tetraploids (Table II). Similar observations have been made in *Brassica* (Howard, 1936). *Scale cereale* (Chin, 1946), *Primula* (Upcott, 1939), *Sorghum* (Magoon and Tayyab, 1968), *Tradescantia* (Anderson and Sax, 1936 and Skrim, 1942) and *Plantago maritima* (Earnshaw, 1942).

Thus, it is evident that in any economic plant where reproductive parts are used, has comparatively poor chance of competing with the diploids either as a strain or as a breeding material. In polyploid breeding, therefore, one can not pitch high hopes on induced tetraploids unless the plants are used for leaves, fodder, other vegetative parts, seedless fruits etc. No wonder induced tetraploid has a good future in forage and fodder crops and horticultural plants like Vines, guava, water melon etc. Taking this clue Mehta and Swaminathan (1955) produced the tetraploid barseem which proved for superior to the diploid counterparts as a forage crops. Similarly triploids sugarbeet has become commercial because of tuberous roots (Peto and Boyes, 1940). Among the cereals, only the autotetraploid

TABLE IV

DISTRIBUTION OF CHROMOSOMES AT ANAPHASE I IN INDUCED TETRAPLOIDS

| No. of PMCs | Distribution | Percentage |
|---|---|---|
| 15 | 14—14 | 15 |
| 10 | 15—13 | 10 |
| 9 | 16—12 | 9 |
| 6 | 17—11 | 6 |
| 5 | 18—10 | 5 |
| 2 | 19— 9 | 2 |
| 3 | 20— 8 | 3 |
| 50 | Laggards, bridges & lagging bivalents | 50 |

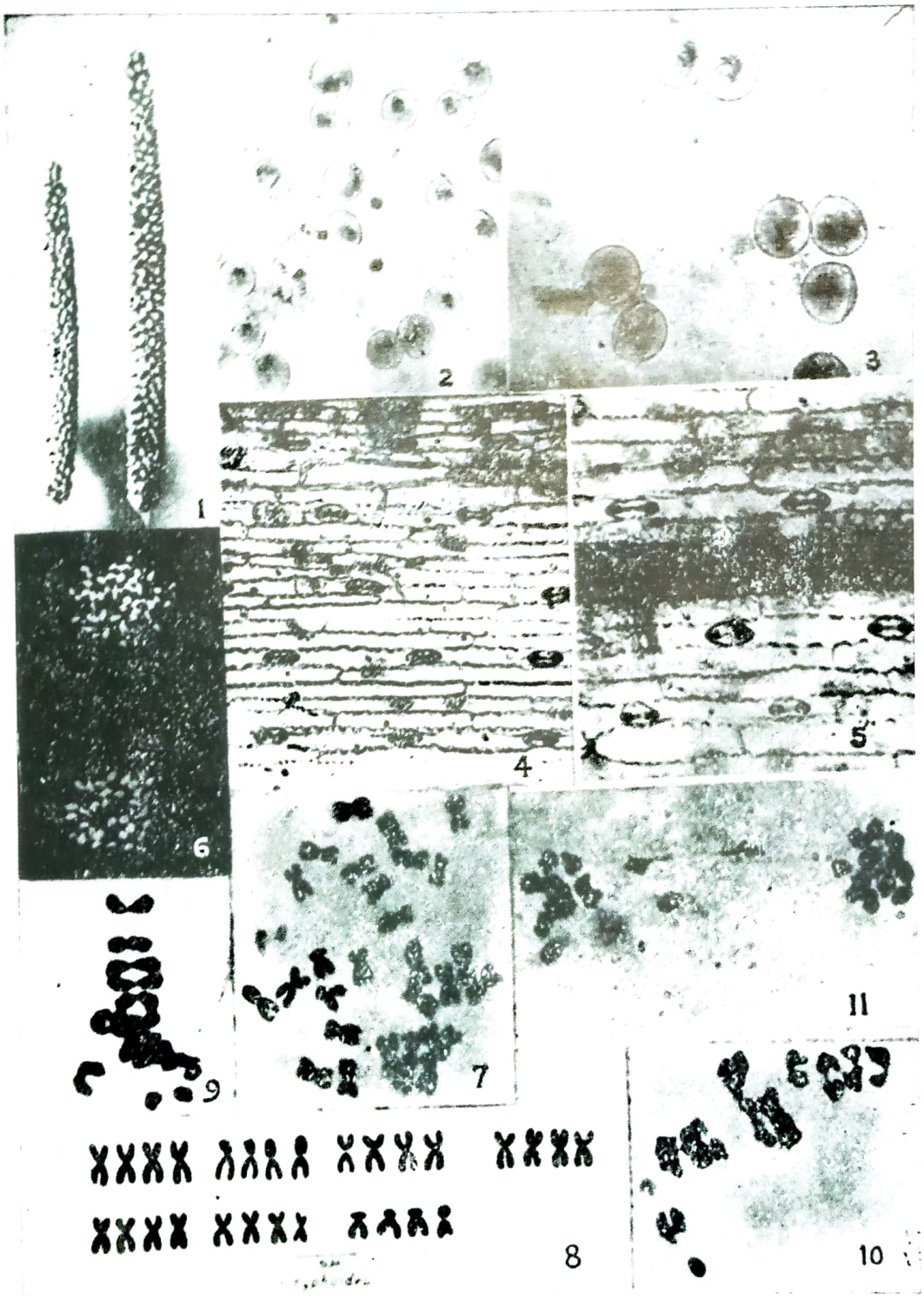
TABLE V

FREQUENCY OF LAGGARDS, BRIDGES AND LAGGING BIVALENTS IN PMCS OF INDUCED TETRAPLOIDS

| Total no. of PMCs analysed 100 | Number of Lagging chromosomes | | | | | | | | Bridges | Lagging bivalents |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | | |
| No. of PMCs | 50 | 10 | 9 | 10 | 6 | 3 | 4 | 1 | 3 | 2 |

is less than 2/3rd whereas in the latter, it is more than 2/3rd. Probably in the former species a certain genetic control reduces quadrivalent frequencies and increases diploidisation. This type of genetic control is also reflected in the direction of chiasma frequencies in the tetraploids (Table II). Similar observations have been made in *Brassica* (Howard, 1936), *Scale cereale* (Chin, 1946), *Primula* (Upcott, 1939), *Sorghum* (Magoon and Tayyab, 1968), *Tradescantia* (Anderson and Sax, 1936 and Skrim, 1942) and *Plantago maritima* (Earnshaw, 1942).

Thus, it is evident that in any economic plant where reproductive parts are used, has comparatively poor chance of competing with the diploids either as a strain or as a breeding material. In polyploid breeding, therefore, one can not pitch high hopes on induced tetraploids unless the plants are used for leaves, fodder, other vegetative parts, seedless fruits etc. No wonder induced tetraploid has a good future in forage and fodder crops and horticultural plants like Vines, guava, water melon etc. Taking this clue Mehta and Swaminathan (1955) produced the tetraploid barseem which proved for superior to the diploid counterparts as a forage crops. Similarly triploids sugarbeet has become commercial because of tuberous roots (Peto and Boyes, 1940). Among the cereals, only the autotetraploid




Figs. 1-11. Fig. 1. Diploid and tetraploid spikes × ½ of the natural size.
Fig. 2. Diploid pollen grains.
Fig. 3. Tetraploid pollen grains.
Fig. 4. Diploid stomata.
Fig. 5. Tetraploid stomata.
Fig. 6. Diploid and tetraploid grains.
Fig. 7. Somatic metaphase (colchiploid, $2n=28$) ×2000
Fig. 8. Karyotype.
Fig. 9. A PMC at metaphase I showing $^{8}II + {}^{3}IV$ ×1250
Fig. 10. A PMC at metaphase I showing $^{2}I + {}^{7}II + {}^{3}IV$ × 1250
Fig. 11. A PMC at anaphase I with a bridge and a laggard × 1250

rye (Ahloowalia, 1967) has proved to be promising perhaps due to rapid nature of diploidization in the species (Muntzing, 1954). But in other crop plants, ploy-ploids are on the whole inferior to diploids due to unstable cytology, non-viable gametes and poor fertility. It needs not however mean that polyploid breeding has no prospect. The very indication that chromosome pairing and behaviour is under genetic control opens up a bright future in chromosome engineering. Chromosomes or sequence of chromosomes can be identified which control normal pairing and subsequent meiotic stages. Such chromosomes can be added to make the species far more efficient in reproductive behaviour. Therefore, stable aneuploids or tetrasomics with specific chromosomes can be established in crop plants. Polyploid breeding in sexually reproducing plants if indirected towards evolving stable aneuploids or tetrasomic appears to have a better future. So far as the induced tetraploid *P. typhoides* is concerned it has not shown any promise either here or in case of Jauhar, 1970, as an economic crop. But because of luxuriant growth and gigas habit, polyploid type can be used for fodder or for hay. Tetraploid may be used for raising useful triploids by crossing with diploids.

## REFERENCES


Ahloowlia, B. S. 1967. Colchicine induced polyploids in rye grass. *Euphytica* **16** : 49-60.

Anderson, E. and K. A. Sax 1936. Cytological monograph of the American species of Tradescantia. *Bot. Gaz.* **97** : 433-476.

Bali, P. N. and S. L. Tandon 1957. Morphological and cytological studies in the diploid and colchicine induced autoteraploid in *Iberis amara* L. *Phyton* **8** : 75-78.

Bali, P. N. and S. L. Tandon 1957. Morphological and cytological studies of the diploid and the colchicine induced autotetraploid in *Iberis umbellata* L. *to Phyton* **12** : 139-146.

Cellarier, R.P. and K. L. Mehra 1958. Determination of polyploidy from herbarium specimens. *Rhodora* **60** : 89-97.

Chin, T.C. 1946. The cytology of polyploid *Sorghum*. *Amer. J. Bot.* **33** : 611-614.

Daker, M. 1967. Cytological studies on a haploid cultivar of Palargonium and its colchicine induced diploids. *Chromosoma* **21** : 250-271.

Dyck, P. L. and T. A. Rajhathy 1965. Desyneptic mutant in *Avena strigosa*. *Canad. J. Genet. Cytol.* **7** : 418-421.

Earnshaw, F. 1942. Experimental taxonomy, V. Cytological studies in sea plantains allied to *Plantago maritima*. *New Phytol.* **41** : 151-164.

Howard, H. W. 1936. The cytology of autotetraploid kale, *Brassica oleracea*. *Cytologia* **10** : 77-87.

Jauhar, P. P. 1970. Chromosome behaviour and fertility of the raw and evolved synthetic tetraploids for Pearl millet, *Pennisetum typhoides* Stapf et Hubb. *Genetica* **41** : 407-424.

Kumar, L. S. S. and A. Abraham 1942. Induction of polyploidy in crop plants. *Curr. Sci.*, **11** : 112-113.

Magoon, M. L. and M. A. Tayyab 1968. Studies on induced polyploids in the genus *Sorghum*. *Nucleus* **11** : 19-33.

Magoon, M. L., D. C. Kooper and R. W. Hougas 1958. Induced polyploids of *Solanums* and their crossability with *S. tuberosum*. *Bot. Gaz.* **119** : 224-231.

Mehta, R. K. and M. S. Swaminathan 1955. Pusa Giant Berseem. *Indian Farming*, Sept.

Mehta, R. K. and M. S. Swaminathan 1957. Studies on induced polyploids in forage crops. *India J. Genet.* **17** : 27-57.

Mishra, U. N. 1968. Ph.D. Thesis, Patna University.

Muntzing, A. 1954. An analysis of hybrid vigour in tetraploid rye. *Heriditas* **40** : 265-277.

Newcomer, E. H. 1941. A colchicine induced tetraploid *Cosmos*. *J. Hered.* **32** : 161-164.

Peto, F. H. and J. W. Boyes 1940. Comparison of diploid and tetraploid sugarbeets. *Canad. J. Res.* 273-282.

Riley, R. and V. Chapman 1958. Genetic control of the cytologically diploid behaviour of hexaploid wheat. *Nature* **182** : 713-715.

Roy, R. P. 1972. PL 480 research report.

Schertz, K. F. 1962. Cytology, fertility and gross morphology of *Sorghum vulgare*. *Canad. J. Genet. Cytol.* **4** :

Sen, N. K. and H. R. Chedda 1958. Colchicine induced tetraploids of five varieties of black gram. *Indian J. Genet.* **18** : 238-248.

SEN, N. K. AND R. V. VIDYABHUSHAN 1960. Studies on the induced polyploids of horse gram (*Dolichos biflorus* L.) *Indian J. Genet.* **20** : 212-222.

SKIRAM, G. W. 1942. Bivalent pairing in an induced tetraploid of *Tradescantia*. *Genetics* **27** : 635-640.

TANDON, S. L. 1950. Colchicine induced polyploidy in spinach. *Curr. Sci.* **19** : 66.

TANDON, S. L. 1955. Colchicine treatment of *Abelmoschus esculentus* (L.) Moench. *Sci. & Cult.* **21** : 39-40.

TANDON, S. L. AND P. N. BALI 1967. Morphological and Cytological studies of the diploid and colchicine induced tetraploid in *Linaria vulgaris*. *Genetica* **29** : 101-109.

UPCOTT, M. 1939. The nature of tetraploidy in *Primula kewensis*. *J. Genet.* **39** : 79-100.