Caryologia
International Journal of Cytology, Cytosystematics and Cytogenetics

Firenze University Press
https://riviste.fupress.net/index.php/caryologia

OPEN ACCESS

Citation: Singh, S. & Kumar, G. (2024). Report of genomic doubling in *Cyamopsis tetragonoloba* (L.) Taub. (Fabaceae): salient features and effects. *Caryologia* 77(3): 63-71. doi: 10.36253/caryologia-2478

Received: Mar 14, 2024

Accepted: Sep 28, 2024

Published: March 25, 2025

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

ORCID
SS: 0000-0001-9670-7286

Report of genomic doubling in *Cyamopsis tetragonoloba* (L.) Taub. (Fabaceae): salient features and effects

Shefali Singh[1,*], Girjesh Kumar[2]

[1] *Department of Botany at K. N. Govt. P.G. College, Gyanpur, Bhadohi, India*
[2] *Plant Genetics Laboratory, Department of Botany, University of Allahabad, India*
*Corresponding author. E-mail: shefalisingh.910@gmail.com

Abstract. Leguminous plants have always been highly valued to meet the nutritional necessities of human. An attempt to for genome multiplication in a legume crop is present in this study. Induced polyploidy as a technique has opened immense future prospects for the agriculture world. In this regard, *in-vivo* autopolyploidization experiment was planned on Cluster bean (*Cyamopsis tetragonoloba* (L.) Taub.). Present study is the successful documentation of the artificial induction of genome doubling by the application of colchicine i.e.C_1 and is the first report of its successful establishment in the next generation (C_2). Colchicine treatment was given to the young seedlings in two different concentrations of 0.2 and 0.4% for three different time durations. Results deciphered that chromosomal complement in case of diploid control plant is 2n=2x=14, whereas in the true polyploids it went on to be 2n=4x=28. Among the total of 148 seedlings that were treated with colchicine solution, 44 putative polyploid plants were monitored based on their distinctive morphological variations. Efficiency of 0.2% concentrations was found to be more than 0.4% concentrations. Palynological and anatomical evidences were also used for ascertaining polyploid organization to the putative plants with increment in size of stomata and pollen grains in the successful autotetraploids. Stomatal size is also an important determinant of ploidy where larger stomata with increase in chloroplast number was a typical feature among tetraploids. Cluster bean is a self-pollinated crop with narrow genetic base, but ploidy manipulation experiment might augment in broadening the genetic diversity. Procurement of large sized flower and seeds is a promising benchmark considering its improved aesthetic value. Autopolyploidization *via* colchicine has bestowed with the splendiferous act that offers prudent significance for diverse fields.

Keywords: Colchicine, chromosome, induced polyploidy, legume, meiosis.

INTRODUCTION

The seemingly proficient pace of morphological up-gradation required several editing at genetic level to augment a more robust genome constitution. One such passage was attained *via* accomplishment of polyploidy has been suggested as a major driving force of plant evolution (Soltis and Soltis

2009). There is ample source of evidence in this relation since many sequenced genomes display the signature of polyploid ancestry (Comai 2005; Yu et al. 2005). Classic studies estimated that 30–50% of angiosperms are polyploids (Stebbins 1950). It is proposed to be the most predominant mechanisms of sympatric speciation in plants (Sattler et al. 2016). The first example of a natural plant polyploid was the gigas mutant of *Oenothera lamarkiana* catalogued by De Vries (Lutz 1907). Lateron, Winkler (1916) recorded first artificial polyploid in *Solanum via* callus regeneration from the surfaces of stem explants and coined the term 'polyploidy' for these types of plant.

Several anti-mitotic agents are known to engineer polyploids artificially such as oryzalin, trifluralin and colchicine. However, in the present study colchicine has been selected. Colchicine is an alkaloid extracted from meadow saffron (*Colchicum autumnale* L.) which binds tubulin dimers *in vitro* and results in the formation of a tubulin–colchicine complex acting primarily to prevent microtubule (MT) assembly (Panda et al. 1995). It has been successfully used to modify the chromosome numbers in diverse plants species including ornamentals, medicinal and cereals.

Cluster bean or Guar (*Cyamopsis tetragonoloba* is a chief leguminous vegetable crop of semi-arid regions of the Indian sub-continent. It is an economically important gum yielding plant and a wonderful green manure crop with magnificient soil replenishing properties. The sterols of guar seeds include campesterol, avenasterol, stigmasterol, sitosterol and traces of Delta-7-avenasterol, stigmast-7-enol, brassicasterol and cholesterol (Mukhtar et al. 2006). The plant contains many important nutrients and phytochemicals such as saponin and flavonoids and is well-known traditional plant used in folklore medicine (Mukhtar et al. 2006; Wang and Morris 2007). Considerable levels of saponin help in normalizing cholestrol levels in body.

Gum yielded from seeds is natural polysaccharide galactomannan rich constituents found effective in osteoarthritis, transdermal drug delivery systems (Murthy et al. 2004). These pharmacological properties and immense economic importance are the compelling forces to enhance the productivity of clusterbean. Attempts to elevate endogenous phytochemical constituents as well as protein content of this excellent nutraceutical plant shall have excellent contribution. This has been the foundation for conceptualising autopolyploidization experiment on cluster bean. Three crucial parameters including cytogenetical, morphological and anatomical were explored for granting confirmatory elucidation regarding successful polyploid induction.

MATERIAL AND METHODS

Plant material

Accessions of seeds of *Cyamopsis tetragonoloba* were procured from regional station of National Bureau of Plant Genetics Resources i.e. Central Arid Zone Research Institute, Jodhpur Rajasthan and among these RGC- 1038 was selected for experimental work.

Agroclimatic conditions of the experimental site

This study was conducted in an experimental cage in Roxburgh Botanical Garden, Department of Botany, University of Allahabad, Prayagraj, UP, India during kharif season (July to November). The geographical location is 25°27'43.01''N, 81°51'10.42''E. Prayagraj lies in sub-tropical climatic zone and receives an annual rainfall of 1027mm where relative humidity is 59%.

Colchicine treatment

Previous literature mentioning standardised protocols for colchicine treatment were referred. Fresh seeds of cluster bean were sown in triplicates in pots. After the emergence of two-cotyledonary stage of seedlings, colchicine treatment ($C_{22}H_{25}NO_6$) was applied on their apical meristem. For this, good quality of sterilized absorbent cotton was utilised for making small spherical balls. Cotton balls dipped in colchicine solution at two different concentrations viz. 0.2% and 0.4%, were placed in between cotyledons carefully for three different time duration of 12 hours (1 day), 24 hours (2 consecutive days) and 36 hours (3 consecutive days), respectively. The plants were covered with earthen pots to prevent evaporation of colchicine solution from the cotton balls. For 24 hours and 36 hours, the treatment was repeated for second and third day also. After completion of each treatment, cotton balls were removed and the growing apical tips were washed thoroughly with distilled water. Plants were carefully monitored in their developing stage in normal field condition.

Morphological studies and anatomical studies

Recording of the various morphological characteristics in the diploid and tetraploids was an important purview of this work. Henceforth, parameters such as plant height, Days to 50% flowering, Days to 50% maturity, leaf length, leaf breadth, cluster per plant, pods per cluster and seed weight were calculated for C_1 and C_2 gen-

Table 1. Colchicine treatment on the apical meristems of seedlings of *Cyamopsis tetragonoloba.*

Concentration	Durations of treatment (hrs)	Number of seedlings	Plants survived	Expected polyploids	Reverted polyploids	Confirmed	
						Number	%
	12	24	23	-	-	-	0
0.2%	24	24	21	8	6	2	8.33%
	36	24	18	15	12	3	12.50
	12	24	17	9	9	-	-
0.4%	24	24	15	10	9	1	4.16
	36	24	12	-	-	-	-

eration. With respect to the control (diploid), morphologically distinct features such as leathery texture and excessive hairy outgrowth were marked, based on this several plants were suspected to be of polyploid organization. The epidermal layer from the abaxial surface of fully expanded leaves was stripped with a razor blade for stomatal study. Stomata of these leaves were observed under microscope by preparing temporary glycerine mounts. Differences in the size of suspected polyploid and diploid plants were taken into account by measuring three parameters i.e. stomatal index, stomatal length and stomatal breadth on micrometer scale in Dewinter Bio-wizard software at 40X resolution. For length and breadth measurement, data from 20 microscopic views were recorded from each slide for diploid and polyploid leaf samples for C_1 and C_2 generation.

Meiotic study

With the arrival of blooming season**,** young floral buds were fixed in Carnoy's fixative (Glacial Acetic Acid and Absolute alcohol in proportion of 1:3, v/v) which were transferred to pure alcohol next day for preservation at 4ºC. These buds were utilised for performing microsporogenic studies where anthers were teased in 2% acetocarmine stain with traces of Iron acetate. Slides were observed under Olympus CH20i at 40X resolution and photography was done using Pinnacle software under Nikon phase contrast microscope. Chromosome counting, as prescribed to be the usual method for ploidy determination (Maluszynska 2003), was performed in the meiocytes of suspected polyploids and diploids was done. Pollen fertility was also calculated as assessment of viability is imperative in relation to the reproductive success of plants. Adequately stained globose pollen grains were marked as fertile against those pollens which appear to be pale yellow with shrunken cytoplasm.

Statistical analysis

Statistical calibration was done using SPSS 16.0 version of software. The means were compared at $p \leq 0.5$ applying Post hoc and Duncan Multiple Range Test (DMRT).

RESULTS

With the help of detailed study, six successful polyploids were isolated on the basis of morphological, cytogenetical and anatomical studies.

Morphological observations

After treatment, an instant retardation in growth was discernible in the seedlings unlike that of the of control set. Emergence of the third leaf was delayed by a week in the treated sets, while in case of control it appeared normally within a span of three to four days after seedling emergence. Survivability was also affected, especially at 0.4% concentration, as some of the seedlings collapsed plausibly because apical meristem was damaged due to cellular necrosis. After initial hindrances, these plants got acclimatized and normalized towards growth and development. However, their rate of growth was still slower than that of the diploid ones which had attained normal plant height and were profusely developed. An array of variations were visible in leaves and this is demonstrated in Figure 1. Leaves were highly deformed and appeared to be invariably concentrated in a whorl at the first node. Leaves were peculiarly leathery, thickened and of fairly large sized as compare to the diploid ones. Large number of trichomes were present on the leaf surface which imparted glabrous texture to the stem and leaf surface, as shown in Figure 1. There were certain plants which initially displayed morphological variations and were prudently examined, however they reverted later on.

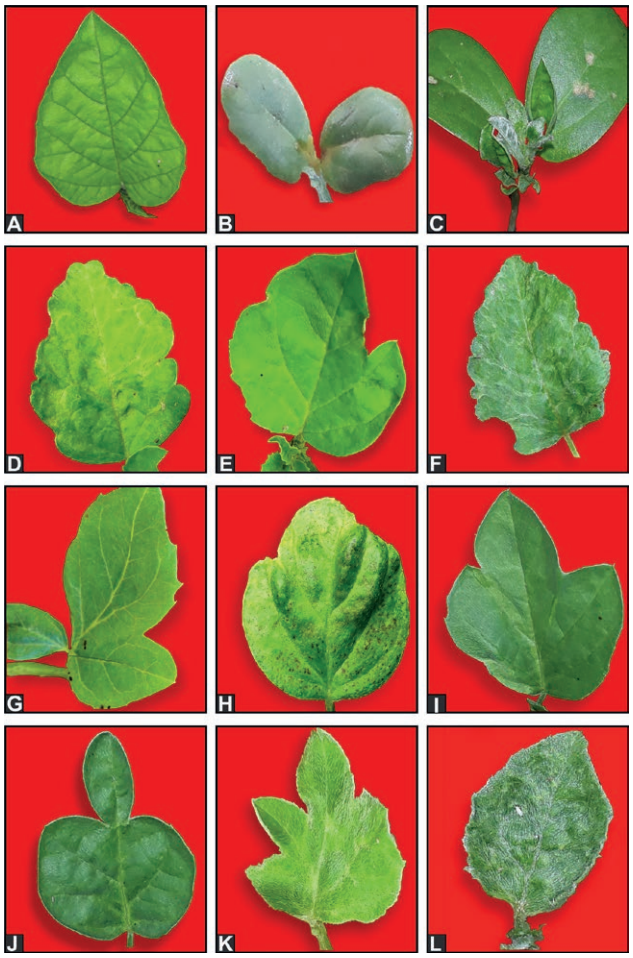

Figure 1. Mutants/variants in leaf shape A: Control; B: Waxy thickened seedling; C: Stunted growth at seedling stage; D: Leathery sinuous leaf; E: Surface extension from one side of leaf; F: Sinulate leaf; G: Leaf fusion among two leaflets of trifoliar leaf; H**:** Leathery coated leaf; I:Triapiculated leaf; J**:** Elliptical leaf protrusion from leaf midrib; K**:** Glabrous assymetrical leaf; L: Tomentosa leaf.

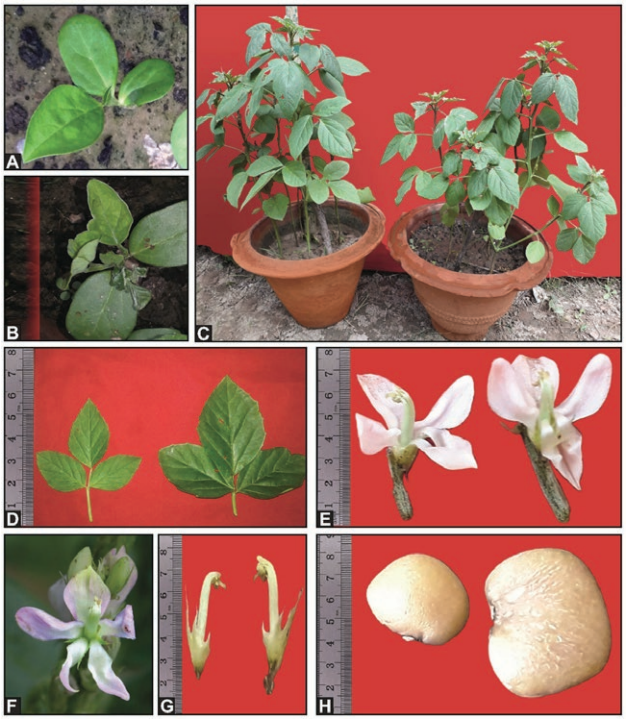

Figure 2. Morphological traits in diploid and autotetraploid plants of Cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.]. A: Seedling of diploid (2n=14); B: Dark green thick texture of putative polyploid seedling; C: Comparative trend of diploid and polyploid plants; D: Leaves of diploid and tetraploid plants; E: Flowers from diploid and tetraploid plants; F: Flower with splitting of petals; G: Reproductive unit of diploid and polyploids; H: Diploid and tetraploid seeds.

With the onset of flowering, several notifiable differences in the reproductive stages were also observed and monitored. Flowers are usually present in clusters but the number of flowers in the cluster was quite less in these putative polyploids; however the size of the flowers (Figure 2E) alongwith the reproductive organs (Figure 2G) was distinctively and conspicuously enlarged with respect to the diploid ones. This behaviour is perceived in relation to the 'gigas' effects of polyploidy. A unique flower with splitting of the two fused petals was spotted (Figure 2F). Days to 50% flowering was recorded, data of which implicits delay in flowering in case of tetraploid plants as mentioned in Table 2. Days to 50% maturity was also delayed by significant margin in tetraploids as compared to the diploids. Seed setting was affected in the polyploids; however the size of seed was enlarged as shown in Figure 2H.

Anatomical observations

Compared to stomata of control plants, stomatal apparatus of several suspected polyploid plants were encountered to be larger, that were surrounded by jumbo sized accessory cells (Figure 3). Number of chloroplast per stomata was also increased. Both stomatal length (22.18±0.38 micrometer) and stomatal breadth (13.45±0.65 micrometer) were remarkably increased in polyploid plants as compare to diploids where calculated stomatal length was 15.93±0.28 micrometer and stomatal breadth was 10.54±0.15 micrometer (Figure 3A and 3B). Data of these parameters is mentioned in Table 2.

Cytogenetical and palynological observations

Previous cytogenetical illustration at mitotic stages has proved that chromosomal complement in *Cyamop-*

Table 2. A comparative analysis of various morphological parameters in diploid and autotetraploid plants of *Cyamopsis tetragonoloba* (L.) Taub. in C_1 and C_2 generation.

Characteristics	Diploid plants (2n=14) (Mean ± SE)	Autotetraploid plants C_1 generation (2n=4x=28) (Mean ± SE)	Autotetraploid plants C_2 generation (2n=4x=28) (Mean ± SE)
Plant height (cm)	86.33±1.91	45.66±1.56	51.70±2.07
Days to 50% flowering	48.66±0.88	65.66±1.45	57.33±1.76
Days to 50% maturity	87.66±1.20	117.33±1.40	113.33±3.84
Leaf length (cm)	7.37±0.52	9.77±0.78	9.60±0.85
Leaf breadth (cm)	4.43±0.26	5.50±0.28	5.87±0.64
Length of Stomatal guard cells (micrometer)	15.93±0.28	22.18±0.38	20.71±0.72
Breadth of Stomatal guard cells (micrometer)	10.54±0.15	13.45±0.65	12.89±0.56
Size of Pollen Mother Cells (micrometer)	16.18±0.74	27.97±1.15	28.08±1.18
Size of Pollen grains (micrometer)	18.93±0.57	33.01±0.83	33.68±0.87
Pollen fertility	97.66±0.88	61.66±0.88	64.33±2.40
Cluster per plant	12.33±1.20	6.33±0.66	6.66±0.33
Pods per cluster	10.00±0.57	4.33 ± 0.88	5.67±0.66
Seed weight (gm)	1.57±0.11	2.37±0.15	2.18±0.14

S.E. = Standard Error.

Table 3. Metaphase I configuration of all induced autotetraploids in *Cyamopsis tetragonoloba* (L.) Taub.

Chromosomal Associations	Percent frequency (Mean ± SE)
8II+2IV+1III+1I	2.97±0.21
7II +3IV+ 2I	2.19±0.1
7II+1IV+1VIII+2I	1.55±0.09
5II+3IV+1VI	0.92±0.24
4II+2IV+1III+1VIII+1I	1.55±0.09
4II+2IV+3III+31	1.25±0.16
3II+2IV+2III +1VII+1I	1.08±0.11
3II+1VIII+1VI+1V+1III	0.77±0.16
3II+1X+1V+1IV+1III	0.95±0.29
3II+2IV+1III+2V+1I	1.25±0.16
2II+2VI+1V+1IV+1III	0.93±0.04
1II+1VI+3IV+1VIII	0.96±0.31
1II+1X+2IV+1V+1III	0.63±0.18
1II+2VI+2V+1IV	0.46 ± 0.02

sis tetragonoloba is 2n=14. Haploid or the base chromosome number of this plant is n=x=7. Figure 4 is the cytological plate which denotes various stages of meiosis in Pollen mother cells of diploid as well as in polyploid cells. Size of PMC was enlarged in case of polyploid cells as it was measured to be 27.97±1.15 micrometer against the diploid cells 16.18±0.74 micrometer. Figure 4A is metaphase I and Figure 4B is anaphase I in case of control. Microsporogenic studies in the suspected polyploids were performed, where diakinesis and metaphase I (Figure 4C) stages revealed that number of chromosomes bivalents was 14. Chromosomal counting was also done at anaphase I where 14:14 poleward separation was recorded (as mentioned in Figure 4D) against the diploid chromosome segregation of 7:7. This provides affirmation to the chromosomal doubling. Several prominent multivalents configurations were recorded, data of which is documented in Table 3. Figure 4E to 4J is the illustrations of multivalent configurations. Laggards (Figure 4N) at anaphase I and unequal separation at anaphase were also recorded. Figure 4M shows 12:16 unequal separation of chromosomes at anaphase I towards opposite poles.

Palynological study was executed to assess viability of pollens in the diploids and polyploids. A noteworthy increment in pollen size was registered in the pollen grains of polyploids. Pollen size of diploid pollen grains was 18.93±0.57 micrometer whereas in polyploids, it was measured to be 33.01±0.83 micrometer. Figure 3C and 3D is diploid and polyploid pollens. Pollen fertility was considerably reduced in polyploids as it declined to a very low percentage of 61.66±0.88 compared to 97.66 ± 0.88% of control.

C_2 generation observations

The plants of C_2 generation were comparatively stronger, resistant, healthier and larger than those of C_1 generation. The morphological traits such as plant

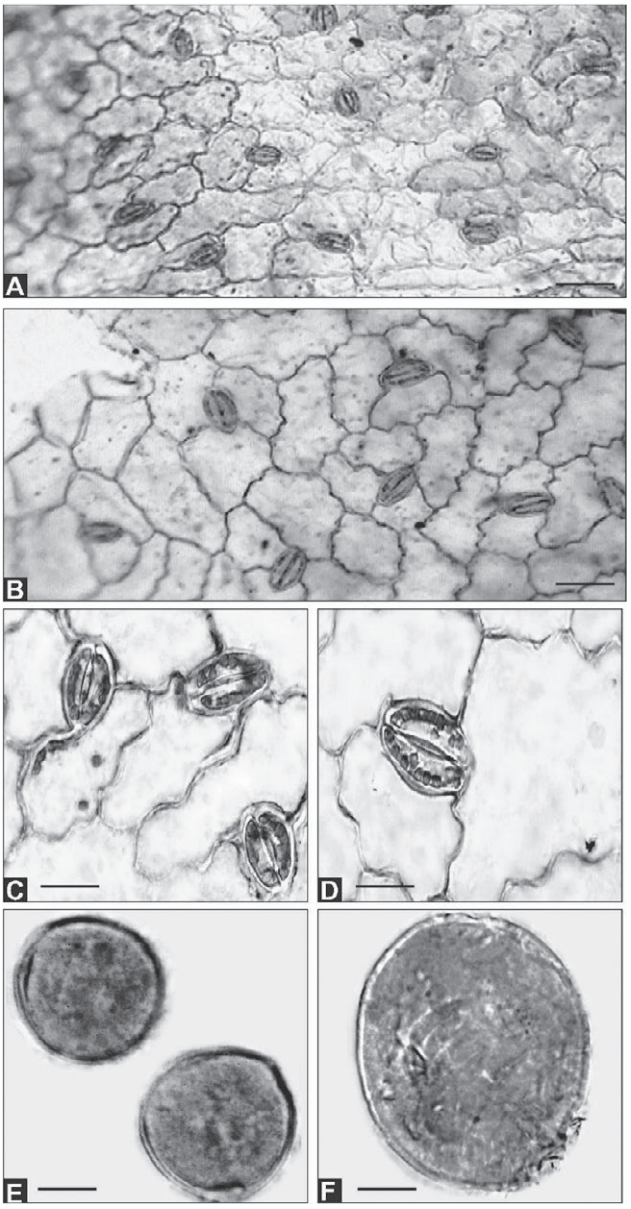

Figure 3. Stomatal and pollen morphology in diploids and autotetraploids of Clusterbean. A: Stomata (diploid) at 10X; B: Stomata (autotetraploid); C**:** Stomata (diploid) at 40X; D: Stomata (autotetraploid at 40X; E: Diploid pollen; F: Tetraploid pollen (40X).

height, length displayed a slight increment while leaf breadth, stomatal guard cell length and breadth displayed a slight decrement in C_2 generation as compared to C_1 generation (Table 2). The morphological parameters such as days to 50% flowering and days to maturity of C_2 generation of colchicine induced autotetraploids were registered as 57.33 and 113.33 days which was considerably earlier than C_1 generation.

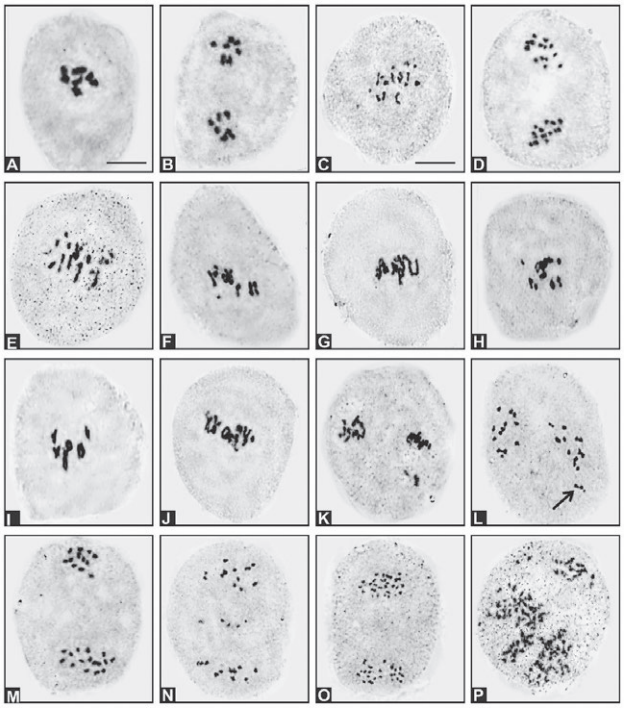

Figure 4. Chromosomal complement in diploids and polyploidy plants. a and b are PMCs of diploid plants- A: Normal metaphase I (seven bivalents); B: Normal anaphase I (7:7 separations), C-P are stages of autotetraploid PMCs- C: Metaphase I with 14 bivalents; D: Anaphase I with 14:14 separations; E-J are multivalents- E: 2II+6I+3III+1IV+1V; F: 4II+2IV+1III+1VIII+1I; G: 1II+1VI+3IV+1VIII; H: 3II+ 2IV+2III+1VII+1I; I: 1X+ 2III+3IV; J: 3II+2IV +1III+ 2V +1I; K: Precocious movement with stickiness at one side at Metaphase II; L: Two precocious at Metaphase II; M: Unequal separation at Anaphase I where 12:16 chromosomes at opposite poles; N: 4 Laggards at Anaphase I; O: Asynchronous division; P: unoriented Anaphase II. Scale bar – diploid cell (16.18 micrometer), polyploid (27.15 micrometer).

DISCUSSION

Polyploidization, in a simple sense, is the heritable condition of possessing more than two complete sets of chromosomes (Comai 2005) which is regarded as an important speciation mechanism for all eukaryotes and has a profound impact on biodiversity dynamics and ecosystem functioning (Ainouche and Jenczewski 2010). Dewey (1980) highlights that each crop species responds differently to polyploidization, depending on their original ploidy level, genome structure, reproduction mode, perenniality and the plant organ for which the crop is cultivated.

Induced tetraploids were conspicuously identifiable among the other diploid population owing to their distinctive morphological features such as vigorousity

in growth, robust nature with thick waxy coated large sized dark green leaves and flowers, excessive trichomes and hairs on stem and leaf surface. This robust nature is explained by the larger number of gene copies which compound into 'gigas' effect to the polyploid (Sattler et al. 2016) which also complements with a higher tolerance to environmental stress (Kermani et al. 2003; Tossi et al. 2022). It appears that there are environmental drivers for coordinated variation in cell size and that changes in genome size can facilitate generic changes in cell size (Jordan et al. 2015). However, gigas effect is not a universal feature of all autopolyploids.

Plants survivability was highly affected due to colchicine and it consistently decreased with the increasing colchicine concentration. Colchicine brings mitotic arrest (Bakar-ates et al. 2018) by inhibiting mitosis by preventing the polymerization of tubulin. This results in a failure of spindle formation, thus, preventing normal chromosomal movement and replication (Kamath et al. 2008). The slower growth rate could be attributed to the lower rate of metabolic activities in the colchitetraploids (Joshi and Verma 2004). Also, rate of cell division was reduced since larger genomes require longer time in cell division, particularly in the S phase (Doyle and Coate 2019).

Stomatal length assessment is regarded to be a reliable and convenient method comparing diploids and polyploids (Jeloudar et al. 2019). Stoma size is associated with CO_2 gain and water discharge in plant photosynthetic and transpiration processes and can be an indicator of ploidy levels (Moghbel et al. 2015). Compare to control, polyploids had enlarged stomatal apertures than those of diploid plants. However there was a reduction in the stomatal density in the tetraploids. Anatomical studies imply that reduction in stoma is a measure to check transpiration discharge. The tetraploid plants had significantly larger stomata and higher chlorophyll content indices, suggesting that the tetraploid plants may have higher photosynthetic and transpiration capacities (Zhang et al. 2018). These adaptive features of a polyploid genome explain their natural invasiveness to extreme environmental conditions and the reason for their greater stabilisation over diploid genome. Number of chloroplast in the stomatal guard cells was also increased in the tetraploids. Polyploid leaves are of dark green texture which might be due to the increase in chloroplast number with quantitative increase in DNA content in tetraploids (Butterfass 1983).

Significance of genomic doubling can be easily realized since several ancient polyploidization events existed at the base of evolution and several of these events gave rise to species-rich groups (Otto 2007). There are several salient features of a genome multiplication which established superlinear advancement of polyploids over their diploid counterparts. For instance, gene redundancy is a very peculiar characteristic of a polyploid cell where redundant copies of the genes have a possible chance for functional diversification (Comai 2005). These redundant set of genes posses the ability of shielding the polyploids from deleterious effects of recessive mutations (Joshi and Verma 2004; te Beest et al. 2012) by providing "buffering actions" in which extra copies of wild-type alleles masks the expression of harmful recessive counterparts. Polysomic inheritance confers higher level of heterozygosity to the polyploid individuals as compare to the diploids (Osborn et al. 2003). Heterozygosity is intimately associated to enhanced vigour.

Transition of vegetative stage into reproductive is associated with activation of floral meristem identity genes. Delay in flowering in polyploids might be related to late triggering of floral responsive genes. Slower growth rates at initial stages results in delay in flowering. Apparently, this delay may also cause reproductive isolation of the neopolyploid. Polyploids are recognised with longer petals, deeper corolla tubes, fewer flowers per inflorescence as found in present work; therefore, attract different assemblages of pollinators compared with diploids. Large sized flowers enhance the aesthetic properties and it may increase pollinator visitation frequencies (Kennedy et al. 2006). This may in turn provide opportunities for diversification in both plant and insect taxa (Nuismer and Thompson 2001). Polyploids also escape pathogenic attack compared to the diploids. Changes in disease resistance genes between polyploids and diploids also point towards altered pathogen resistance (Innes et al. 2008).

The plausible causes of the chromosomal abnormalities encountered in polyploids complement is perhaps the struggle among the doubled chromosome number in the neo-polyploid. Diploid PMCs oblige seven bivalents of the cell perfectly but the tetraploid PMCs have to accommodate more bivalents. This chromosomal doubling setsup an inter-repulsive hindrance among the chromosomes which reciprocates into aberrant meiosis. Occurrence of multivalents particularly quadrivalents was conspicuous in varying frequencies in the present tetraploids, since the latter are derived from a single genome resulting in four homologous sets of chromosomes in tetraploids (Stace 1980). These multivalents create abnormal patterns during anaphase such as '3:1' or '2:1 plus one laggard (Comai 2005). Such type of improper segregation results in formation of abnormal gametes with unbalanced ploidy that lead to aneuploids and sterility.

A noteworthy feature recorded during palynological assessment was the increment in pollen grain diameter

among the polyploids. However, there was a substantial deceleration in pollen viability in the tetraploids, as also reported in *Pinellia ternata* (Thunb.) (Liu et al. 2012). Parthasarathy and Rajan (1953) have advocated that chromosome doubling in the tetraploid may upset the balance of polygenes or modifying genes which may probably control the sterility. As a consequence of chromosomal aberrations, some sort of sterility takes place resulting to lower seed yield which is a barrier for the inheritance of polyploid (Kumar and Dwivedi 2017). Reduction in seed yield is also an after-effect of the pollen sterility; since reproductive success largely relies on the viability of gametes. However, the seed size and weight were increased.

Change in ploidy may also have substantial effect in altering quantity and quality of secondary metabolites. Polyploidization can be artificially induced to increase the production and/or improve the quality of important medicinal compounds, such as pharmaceuticals and aroma chemicals (Dhawan and Lavania 1996). Medicinal aromatic polyploids *viz. Carum carvi* L. (Dijkstra and Speckman 1980), *Ocimum kilimandscharicum* Gürke (Bose and Choundhury 1962) had increased terpene levels and elevated essential oil concentration. These changes in phytochemical composition might be associated with difference in triggering of metabolic cascade in the polyploid cell.

Science of polyploidization has been adopted by researchers and plant breeders for attaining superior genotypes. Natural autopolyploidization is well proven to be proximal drivers in specie diversification and differentiation whereas inception of *in vivo* polyploidization bears the potential to revolutionize the face of agricultural and pharmaceutical areas. Leguminous crops such as cluster bean has narrow genetic base, but ploidy manipulation experiment might augment in broadening the genetic diversity. Genomic doubling opens a new passage for neo-polyploids to evolve and diversify, but there is a need to further the level of knowledge on the mechanism behind this as it is still not very clearly understood. Gigas effect and robust nature of established polyploids are important characteristics that might confer environmental tolerance to the neo-polyploid. Shorter plant height, as observed in the present documentation, can act as a boon for plants with lodging issues. Increased flower size is also significant attribute that elevates the aesthetic properties which is of profound interest in floriculture. These traits had played pivotal role in past and may also have crucial influence in future on plant diversification and invasion to hostile realms.

ACKNOWLEDGEMENT

Authors express gratitude towards the lab members of Plant Genetics Laboratory for their guidance and support. One of the author who is Assistant Professor at KN Govt. PG College also acknowledges her Department members for their cooperation.

AUTHOR'S CONTRIBUTION

Prof. Girjesh Kumar gave expert guidance and also did editing and corrections in the manuscript. Dr. Shefali Singh designed the experiment, conducted it and wrote the manuscript.

REFERENCES

Ainouche ML, Jenczewski E. 2010. Focus on polyploidy. The New Phytologist. 186:1-4.

Bakar-Ateş F, Özmen N, Kaya-Sezginer E, Kurt EE. 2018. Effects of colchicine on cell cycle arrest and MMP-2 mRNA expression in MCF-7 breast adenocarcinoma cells. Turk Hij Den Biyol Derg. 75:239-244.

Bose RB, Choudhury JK.1962. A comparative study of the cytotaxonomy, palynology and physiology of diploid and polyploid plants from *Ocimum kilimandscharicum* Guerke and their yield of raw material and volatile contents. Caryologia 15:435–453.

Butterfass TH.1983.A nucleotypic control of chloroplast reproduction. Protoplasma. 118:71-74

Comai L. 2005. The advantages and disadvantages of being polyploid. Nature Review Genetics. 6:836-846.

Dewey DR. 1980. Some applications and misapplications of induced polyploidy to plant breeding. In: Lewis WH (ed) Polyploidy: biological relevance. Plenum Press, New York, pp: 445–470.

Dhawan OP, Lavania UC. 1996. Enhancing the productivity of secondary metabolites via induced polyploidy: a review. Euphytica. 87:81-89.

Dijkstra H, Speckman GJ. 1980. Autotetraploidy in caraway (*Carum carvi* L.) for the increase of the aetheric oil content of the seed. Euphytica.29:89–96.

Doyle JJ, Coate JE. 2019. Polyploidy, the nucleotype, and novelty: the impact of genome doubling on the biology of the cell. Int J Plant Sci. 180:1–52.

Innes RW, Ameline-Torregrosa C, Ashfield T, Cannon E, Cannon SB, Chacko B, Chen NW, Couloux A, Dalwani A, Denny R, et al. 2008. Differential accumulation of retroelements and diversification of NBLRR disease resistance genes in duplicated regions fol-

lowing polyploidy in the ancestor of soybean. Plant Physiol.148:1740–1759.

Jeloudar NI, Chamani E, Shokouhian AA, Zakaria RA. 2019. Induction and identification of polyploidy by colchicine treatment in *Lilium regale*. Cytologia.84:271-276.

Jordan GJ, Carpenter RJ, Koutoulis A, Price A, Brodribb TJ. 2015. Environmental adaptation in stomatal size independent of the effects of genome size. New Phytol. 205: 608-617.

Joshi P, Verma RC. 2004. High frequency production of colchicine induced autotetraploids in faba bean *(Vicia faba L.)*. Cytologia. 69:141-147.

Kamath A, Mehal W, Jain D. 2008). Colchicine-associated ring mitosis in liver biopsy and their clinical implications. J Clin Gastroenterol. 42:1060.

Kennedy B, Sabara HA, Haydon D, Husband BC. 2006. Pollinator-mediated assortative mating in mixed ploidy populations of *Chamerion angustifolium* (Onagraceae). Oecologia. 150:398-408.

Kermani MJ, Sarsan V, Roberts AV, et al, (2003) Oryzalin induced chromosome doubling in *Rosa* and its effect on plant morphology and pollen viability. Theor Appl Genet. 107:1195–1200.

Kumar G, Dwivedi H. 2017. Induced Autotetraploidy in *Trachyspermum ammi* (L.) Sprague (Apiaceae). Cytol Genet.51:391–400.

Liu Y, Hui RK, Deng RN, Wang JJ, Wang M, Li ZY. 2012. Abnormal male meiosis explains pollen sterility in the polyploid medicinal plant *Pinellia ternata* (Araceae). Genet Mol Res. 11:112-120.

Lutz AM. 1907. A preliminary note on the chromosomes of *Oenothera lamarkiana* and one of its mutants, *O. gigas*. Science. 26:151–152.

Maluszynska J. 2003. Cytogenetic tests for ploidy level analyses – chromosome counting. In: Doubled Haploid Production in Crop Plants: A Manual, Maluszynski, M., Kasha, K.J., B.P. Forster and I. Szarejko, pp. 391-395, Kluwer Academic Publishers, ISBN 1- 4020-1544-5, Dordrecht.

Moghbel N, Borujeni MK, Bernard F. 2015. Colchicine effect on the DNA content and stomata size of *Glycyrrhiza glabra* var. glandulifera and *Carthamus tinctorius* L. cultured *in vitro.* J Genet Eng Biotechnol. 13:1–6.

Mukhtar, HM, Ansari SH, Bhat ZA, Naved T. 2006. Anti-hyperglycemic activity of *Cyamopsis tetragonoloba* Beans on blood glucose levels in alloxan-induced diabetic rats. Pharm Biol. 44:10-13.

Murthy SN, Hiremath SRR, Paranjothy KLK. 2004. Evaluation of carboxymethyl cluster bean films for the formulation of transdermal therapeutic systems. Int J Pharm. 272: 11-18.

Nuismer SL, Thompson JN. 2001. Plant polyploidy and non-uniform effects on insect herbivores. Proc Royal Soc B 268:1937–1940.

Osborn TC, Pires JC, Birchler JA, et al. 2003. Understanding mechanisms of novel gene expression in polyploids. Trends Genet.19: 141–147.

Otto S. 2007. The Evolutionary Consequences of Polyploidy. Cell. 131: 452-462.

Panda D, Goode BL, Feinstein SC, Wilson L. 1995. Kinetic stabilization of microtubule dynamics at steady state by tau and microtubule-binding domains of tau. Biochemistry (Washington). 34: 11117–11127.

Parthasarathy N, Rajan SS 1953. Studies on the fertility of autotetraploid of *Brassica campestris* var. toria. Euphytica. 2: 25-32.

Sattler MC, Carvalho CR, Clarindo WR. 2016. The polyploidy and its key role in plant breeding. Planta. 243: 281–296.

Shyale S, Chowdary K, Krishnaiah Y, Bhat NK. 2006. Pharmacokinetic evaluation and studies on the clinical efficacy of cluster bean gum-based oral drug delivery systems of albendazole and albendazole-β-cyclodextrin for colon targeting in human volunteers. Drug Development Res. 67: 154-165.

Soltis P, Soltis D. 2009. The role of hybridization in plant speciation. Ann Rev Plant Biol. 60: 561–588.

Stace CA. 1980. Plant Taxonomy and Biosystematics. Edward Arnold, London.

Stebbins GL. 1950. Variation and evolution in plants. New York, NY Columbia University Press.

te Beest, M, Le Roux JJ, Richardson DM, Brysting AK, Suda J, Kubesova M, Pysek P. 2012. The more the better? The role of polyploidy in facilitating plant invasions. Ann Bot. 109: 19–45.

Thompson JN, Merg KF. 2008. Evolution of polyploidy and the diversification of plant–pollinator interactions. Ecology. 89: 2197–2206.

Tossi VE, Martínez Tosar LJ, Laino LE, Iannicelli J, Regalado JJ, Escandón AS, Baroli I, Causin HF, Pitta-Álvarez SI. 2022. Impact of polyploidy on plant tolerance to abiotic and biotic stresses. Front Plant Sci. 13 869423.

Wang ML, Morris JB. 2007. Flavonoid content in seeds of guar germplasm using HPLC. Plant Genet Resour: Characterization Util. 5: 96.

Winkler H. 1916. Uber die experimentelle erzeugung von pflanzen mit abweichenden chromosomenzahlen. Zeits Bot 8: 417–531.

Yu J *et al.* 2005. The Genomes of *Oryza sativa*: a history of duplications. PLoS Biol. 3: 266-281.

Zhang H, An S, Hu J, Lin Z, Liu X, Bao H, Chen R. 2018). Induction, identification and characterization of polyploidy in *Stevia rebaudiana* Bertoni. Plant Biotechnol J. 35: 81-86.