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RESEARCH ARTICLE

Induced polyploidization in buckwheat (*Fagopyrum esculentum* Moench)

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Abstract

The significance of polyploidy in the evolutionary process of plants history is well documented. In this concern, seed priming with colchicine as well as the cotton-swab method for polyploidy induction in plants becomes much acquainted in cytogenetic study. The aim of present study was to achieve chromosomal doubling by the application of colchicine to apical meristem of young seedlings. The young seedlings were treated with three different concentrations of colchicine (0.2, 0.4 and 0.6%) for durations of 12, 24 and 36 h each. The colchiploidy with highest proportion approximately 50% with 2n=4x=32 was found at 0.2% concentration for 24 and 36 h. Autotetraploid enduring plants were identified with morphological and cytological variations such as thickness of leaf, larger stomata with low density, larger pollen, large flower etc. Buds were selected from these plants for the cytological study. Cytological analysis including chromosome counting demonstrated that the chromosome number was doubled. The confirmation of autotetraploid plants was achieved by this technique. Tetraploid plants were grown up to maturity and the harvested seeds were sown to establish the second generation which may be used in future breeding programme.

Keywords: Autotetraploids**,** chromosome doubling, colchicine, *Fagopyrum esculentum,* meiosis

Introduction

Common buckwheat, *Fagopyrum esculentum* Moench of the family Polygonaceae is the oldest and most important food used as pseudo-cereal crop. Buckwheat achieved popularity for its higher food production. But in the recent years, the crop production has reached at declining stage because of the competition with other major cereal crops. For this, a special attention is being required to increase its yield through genetic improvement (Kreft et al. 2020). Common buckwheat has a promising future on world wide scale due to recent discoveries on its nutritional values (Cawoy et al. 2009). Since, the crop offers numerous medicinal properties hence, to achieve better responses, experiment was conducted to induce polyploidy. It is a goal in the breeding of some plant species since it is accompanied by implemented biomass production of crop plants (Schubert et al. 1991). For the implementation, breeding programmes have been employed with the aim of obtaining fertile varieties. Such programmes have included artificial selection from natural population, mutagenesis and genetic improvement (Zhou et al. 1993).

Crossing of diploid and tetraploid is one of the ways for achievement of polyploid individuals. Some sterile individuals of common buckwheat could be obtained through this alternative approach. There are published reports of hexaploid buckwheat (*F. tibeticum*, 2n=6x=48) from the interspecific cross between diploid *Fagopyrum esculentum* and tetraploid *Fagopyrum homotropicum* (Tian et al. 2009), but there are no reports on the availability of autotetraploids of *Fagopyrum esculentum*. Present work thus holds significance of polyploidization in cultivable specie of buckwheat. However, majority of species are diploids with 16 chromosomes which are natural and hybrid but spontaneous polyploidy is rare, at best (Chrungoo and Chettry 2021). Thus, artificial induction of tetraploid line is a demanding method. For the execution of auto-polyploids

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a well-known chemical, colchicine is used which is spindle inhibitor and responsible for the chromosomes doubling disrupting the cell division and preventing microtubule polymerization and polar movement of chromosome at anaphase (Liu et al. 2007). Henceforth, its application is envisaged for creating useful tetraploid buckwheat lines with better agronomic traits.

Polyploidy is widely acknowledged as an important and amazing evolutionary event of adaptation and speciation in the evolution of higher plants (Ramsey and Schemske 1998). It is one of the major targets of breeding programme which has been used to improve the crop cultivars. Blackeslee and Avery (1937) discovered the ability of colchicine for chromosome doubling. Polyploidy refers to the presence of more than two complete sets of chromosomes per nucleus, which has been considered as a ubiquitous phenomenon (Soltis et al. 2009). It involves a series of molecular and physiological adjustments and plays a vital role in plant evolution and diversification, at temporal scales ranging from ancient to contemporary, and with profound impacts at scales ranging from molecular to ecological (Adams and Wendel 2005). Polyploid individuals may exhibit larger organs as compared to their diploid counterpart, such as roots, leaves, fruits, flowers, pollen, stomata cells, trichome and seeds (Talukdar 2010; Kumar and Dwivedi 2014).

Polyploidy induction can be used as a means to create and select new and better breeds for the future use (Kushwah et al. 2018). It has long been suggested to enjoy a variety of capabilities that transgress those of their diploid progenitors, such as adaptation to environmental extremes (Muntzing 1936; Love and Love 1949; Stebbins 1950), better adaptability and resistance, wider ecological niche and successful colonization of a greater range of different habitat, formation of extreme phenotypes, and increased vigor (Van de Peer et al. 2009). Colchicine is an alkaloid obtained from the roots of the meadow saffron (*Colchicum autumnale*). It hampers the development of the nuclear spindle. It 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). As a result, proper separation and segregation of chromosomes is affected and the duplicated chromosomes remain dispersed as such in the cell which leads to the doubling of entire chromosome number that increases the volume of cell. The present investigation was carried out to develop tetraploid (4x) *Fagopyrum esculentum* Moench through the *in vivo* colchicine treatment and explore the potential of artificial polyploidy as a source of induced variation. The study also compares morphological traits of diploid controls and *in vivo* induced tetraploids.

Materials and methods

Plant material

The inbred seeds of *Fagopyrum esculentum* (Variety- VL-7) were obtained from National Bureau of Plant Genetic Resources, Shimla, Phagli for experimental work.

Agroclimatic conditions of experimental site

The present experimental setup has been performed in the province of Roxburgh Botanical Garden, Department of Botany, University of Allahabad, Prayagraj, U.P. during the winter season of 2014 to 2017. The location of performed experiment is 25°27′43.01″N, 81°51′10.42″E. Prayagraj is situated 98 m above mean sea level and falls under sub-tropical climatic zone. The average rainfall in this area is 95.9 mm. The relative humidity varies from 45 to 100%. Alluvial soil is found in this region and textural class is sandy loam. The soil is low in organic matter.

Colchicine treatment

The inbred seeds of Variety VL-7 were sown in the experimental pot in three replicates along with control set. After three to five of sowing days, germination started. The aqueous solution of colchicine of different concentrations was prepared. A range of alternative concentrations of colchicine was applied *viz.* 0.2%, 0.4% and 0.6% for different time periods as 12, 24 and 36 hours each along with recovery period of 12 hours. Seedlings were treated at two cotyledonary stage before the emergence of third leaf. A dipped cotton plug in different concentrations of colchicine solution was applied on the apical vegetative seedlings. After treatment, the seedlings were then covered with earthenware pots as a measure to check evaporation. Treatment was made for different durations. After the completion of treatment, cotton plug was removed and seedlings were washed through tap water.

Morphological assessment of seedlings at preliminary stage

A large number of colchicine treated seedlings were carefully examined at preliminary stages. Firstly, an initial identification of tetraploid plants was conducted on the basis of stomatal size. Some morphological features like slow growth, thickness of leaf and condensed form were helpful for the screening of tetraploid plants. 2-3 leaf samples of such plants were taken and peeled off the epidermal layer from the abaxial surface of expanded leaves and placed on slide and stained then covered with a cover slip and observed under microscope using 40X resolution vision software. Leaf sample of control plants was also studied. The size of stomata was calibrated with an occular scale and stomatal density was also observed. The density and size of stomata of diploid and tetraploid plants were compared. For the study, 15 stomata were measured in each slide for the

Table 1. Effect of colchicine treatment in the apical meristem of seedlings of *Fagopyrum esculentum* Moench

Colchicine concentration (%)	Treatment durations (hrs)	No. of seedlings treated	Survival rate (%)	No. of plants examined in cytological analysis	Ploidy level		Polyploidization efficiency (%)
					Diploid (2n)	Autotetraploid (4x)	
Control	-	15	100.00	10	10	-	-
	12	15	60.00	8	7	1	6.66
0.2	24	15	46.66	9	6	3	20.00
	36	12	50.00	9	4	5	41.66
	12	15	46.66	7	5	2	13.33
0.4	24	14	50.00	7	6	-	-
	36	15	40.00	4	4	-	-

confirmation of tetraploid plant.

Cytological assessment

Cytological analysis was conducted for the verification of chromosome no. and the confirmation of tetraploid plants. Young floral buds of suitable size were fixed in Carnoy's fixative I (Ethanol3: Glacial Acetic Acid 1) and then transferred in 90% alcohol after 24 hours as a preservation for the meiotic study. Anthers were excised from the young floral buds and kept on a slide. Buds were teased and stained in 2% acetocarmine, followed by squash preparing slides observed under a microscope. At least 10 slides of young buds were observed for each seedling. The size of pollen grains was also measured with an occular scale and the pollen fertility was evaluated by acetocarmine stainability test.

Statistical analysis

Three replicates of each attributes were taken for this study. Statistical analysis was performed using SPSS 16 Software. Coefficient of variation applied for measurement of Relative dispersions in morphological parameters was also calculated. Actual mean and standard error were also calculated.

Results and discussion

Effect of colchicine on autotetraploidy induction

The present investigation stated that the seedling treatment through cotton swab method was practically effective for the induction of autotetraploid in *Fagopyrum esculentum* Moench. An autotetraploid was induced at different concentrations of solution and duration of application. The highest survival rate (60%) was recorded at a treatment of 0.2% colchicine given for duration of 12 hrs. Therefore, 0.2% colchicine for 12 hrs was found to be effective as the least lethal dose. However, the highest frequency of induced polyploids was recorded as 41.66% at a dose of 0.2% colchicine given for duration of 36 hrs. Hence, it was regarded as the optimum dose. Colchicine treated plants at 0.4% for 12 h showed successful induction of autotetraploids with a polyploidization efficiency of 13.33% but no seed setting was observed after survival to maturity. Total eleven plants were recorded as autotetraploids at various concentrations which were found to be 1 in 0.2% (12 hrs.), 3 in 0.2% (24 hrs.), 5 in 0.2% (36 hrs.) and 2 in 0.4% (12 hrs.). The above mentioned effects have been summarized in Table 1. Among the 101 colchicine treated seedlings, 11 plants were identified as autotetraploids on the basis of morphological as well as cytological parameters while remaining treated seedlings died or reverted back to normal diploid condition.

Polyploid plants can arise spontaneously in nature by several mechanisms, including meiotic or mitotic failures and fusion of unreduced (2n) gametes (Comai 2005). It is accepted that all seed plants have experienced at least one round of whole genome doubling in their evolutionary history, characterizing a paleopolyploidy ancestry (Renny-Byfield and Wendel 2014). Artificial induction is conveniently achieved by usage of chemical colchicine (Blakeslee and Avery 1937). It has been considered as a method for increasing production potential of plant secondary metabolites (Omidbaigi et al. 2010). Aut ot et raploidy is valuable for plant breeders and can be used in breeding programme, to influence the level of heterozygosity which is of profound importance for creating variations. It may be due to polysomic inheritance and the possibility of outcrossing occurrence (Osborn et al. 2003). Many opportunities available through these developing auto-tetraploids include developing sterile cultivars, flower enlargement, greater resistance to pests, greater nutrient, increasing vigour and tolerance to biotic and abiotic stresses. Furthermore, traits such as increment in number of trichomes and reduction in stomatal frequency indicate enhanced drought tolerance capacity of autotetraploids. This may correlate the effect of genome dosage to gaseous exchange and water relation which enhances the potential of polyploids to tolerate the drought stress (Dwivedi and Kumar 2017).

Induction of autotetraploids is envisioned to meet the increasing demands of buckwheat regarding its nutraceutical and medicinal properties. The autotetraploidy was confirmed in plants by growth rate, morphological parameters, studies of stomata and pollen grain size as well as by cytological analysis. In the present investigation, the seedling treatment method of polyploidization in 36 hours

Table 2. Morphological characteristics of diploid and autotetraploid plants of *Fagopyrum esculentum* Moench

Characters studied	Diploid (2n=16)	Autotetraploid (2n=4x=32)	
		C1 generation	C2 generation
Plant height (cm)	71.20 ± 0.91	40.03 ± 0.57	45.60 ± 0.64
Length of branches (cm)	11.94 ± 0.48	7.31 ± 0.41	27.7 ± 1.15
Internodal distance of main stem (cm)	7.15 ± 0.26	5.24 ± 0.20	6.20± 0.17
Length of leaf (cm)	6.70 ± 0.23	3.79 ± 0.30	4.51± 0.23
Breadth of leaf (cm)	5.03 ± 0.49	3.20 ± 0.19	4.22± 0.17
Leaf area (cm^2)	23.75 ± 0.26	13.87 ± 0.24	14.66 ± 0.21
Thickness of leaf (mm)	0.67 ± 0.12	1.92 ± 0.19	2.11 ±0.15
Fresh weight of leaf (mg)	4.65 ± 0.12	9.26 ± 0.16	9.54± 0.11
Length of stomata (μm)	20.30 ± 0.18	42.66± 0.23	43.50 ± 0.59
Breadth of stomata (μm)	15.71 ± 0.11	33.70± 0.13	34.38± 0.52
No. of stomata per unit area (nmm^{-2})	220.00± 1.15	128.00 ± 2.02	127.00 ± 2.89
Total no. of pollen grains	192.00 ± 1.70	147.00 ± 1.29	149.00 ± 2.77
Size of pollen grains (μm)	43.28 ± 0.15	62.23± 0.13	63.46± 0.74
Pollen fertility (%)	92.71 ± 0.14	55.63 ± 0.09	57.26 ± 0.74
Seed setting (%)	76.97 ± 0.70	57.34 ± 0.19	60.33 ± 1.00
Average weight of 50 seeds (gm)	3.34 ± 0.22	6.53 ± 0.19	6.76± 0.16
Days to 50% flowering	45.00±1.58	65.00±1.73	61.00± 1.15
Days to maturity	99.00±0.57	124.00±1.37	118.00±2.31

Table 3. Chromosome configurations at metaphase I of induced autotetraploid plants (C_1 and C_2 generations) of *Fagopyrum esculentum* Moench

Chromosomal configurations	Frequency of PMC's at Metaphase I (%)
6I + 7II+2VI	5.00
8I+5II+2III+2IV	7.5
3I+8II+2IV+1V	12.5
6I+6II+2III+2IV	5.00
4I+7II+2IV+1VI	20.00
2I+5II+2III+1IV+2V	17.50
3II+1III+3IV+1V+1VI	10.00
2I+5II+2IV+2VI	12.50
3I+4II+1III+3IV+1VI	7.50
8II+2III+1IV+1VI	2.50

with 0.2% colchicine was very successful since 41.66% of the colchicine treated seedlings were autotetraploid.

Morphological observations

Autotetraploid plants became morphologically distinct from the diploid ones and were easily identified in various ways. The development and growth rate of polyploid plants were very slow and first flowering took longer time in comparison to the diploid counterparts. The basal two leaves of these plants became thick, waxy and distorted in shape, dark green and the surface also became rough and glabrous while upper newly formed leaves fulfilled the above characteristics but small in size as compared to basal leaves. The cotyledonary leaves show gigas effect and arrested growth for few days. However, the diploid plants displayed the normal growth pattern. After few days of arrested growth, the shoots aroused from the tip and showed deformity. Later on, the deformed leaves became normal. All phenotypic traits of autotetraploid and diploid plants such as length of branches, internodal distance, leaf length and breadth, leaf area, leaf thickness, fresh weight of leaf were recorded to have a significant increment in its mean value. The above mentioned observations have been summarized in Table 2. Plant height of tetraploid was much declined as compared to diploid which was found as 40.03 cm and comparative trends of autotetraploid and

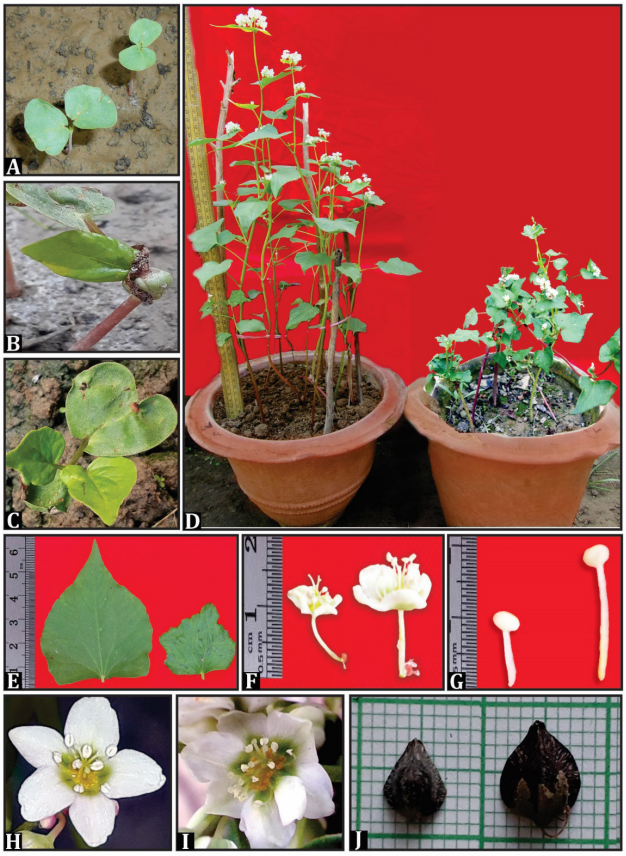

Fig. 1. Induced Autotetraploid in Fagopyrum esculentum Moench: A = Seedling (Control), B = Swollen node at seedling stage, C = Two basal leaves at one node where one is smaller than other one, D = Diploid plant (left side), Autotetraploid plant (right side), E = Diploid leaf (left side), Autotetraploid leaf (right side), F = Diploid flower (left side), Autotetraploid flower (right side), G = Diploid anther (left side), Autotetraploid anther (right side), H = Diploid flower with five tepals, I = Autotetraploid flower with seven tepals and J = Diploid seed (left side), Autotetraploid seed (right side)

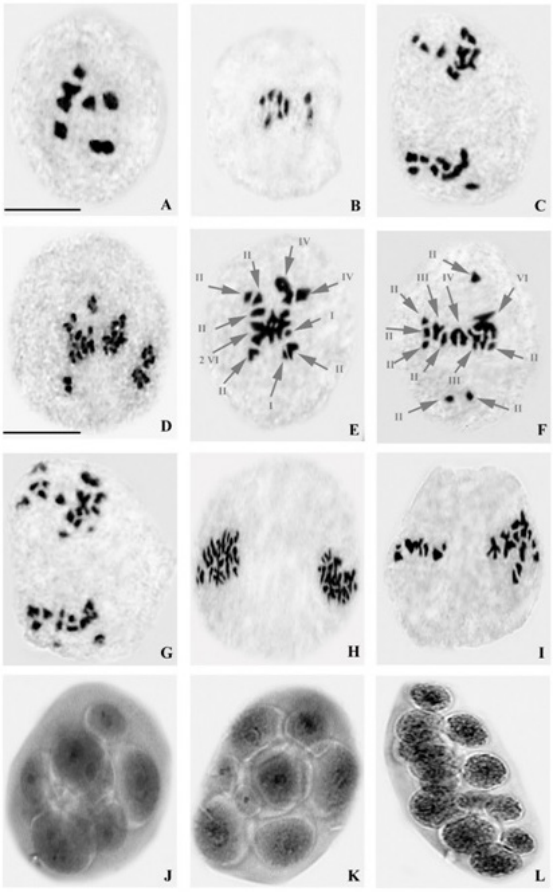

Fig. 2. Induced autotetraploidy in Fagopyrum esculentum Moench (A-C: Diploid, D-L: Autotetraploid). A = Normal PMC showing 8 bivalents at diakinesis, B = Normal Metaphase I (2n=16), C = Normal Anaphase I (8:8 separations), D and E = PMC showing autotetraploid Metaphase I, F = PMC showing autotetraploid Metaphase I with 3 precocious chromosomes, G = PMC showing autotetraploid Anaphase I (16:16 separations), H = PMC showing autotetraploid Metaphase II, I = Unequal segregation of chromosomes at Metaphase II, J = Hexad, K = Octad and L = Polyad [Scale bar: Diploid- 8.62µm, Autotetraploid- 10.92µm]

diploid has been illustrated in Fig. 1 Further, daysto 50% flowering and days to maturity were delayed by 20-25 days in autotetraploids during C_1 generation. Autotetraploids exhibited reduction in flowers as compared to diploids and it persisted for longer duration. The mean value of seed setting in diploid was recorded as 76.97% which was much reduced in autotetraploids i.e. 57.34% in C_1 generation (Table 2). 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 height, length and breadth of leaf, internodal distance etc. displayed a slight increment 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 autotetraploids were registered as 61 and 118 days, respectively which were lower than that of C_1 generation.

A part from this, some morphological mutants/variants were also observed in induced polyploids like leaf, flower and seed mutants/variants. Cotyledons showed disturbed phyllotaxy with swollen node i.e. cotyledonary leaf arose at one node in one direction (Fig. 1B) and basal leaf deformity was also observed i.e. the basal leaves became thick, waxy, distorted in shape, dark green and the surface also became rough and glabrous while the upper newly formed leaves were smaller in size as compared to the basal ones (Fig. 1C). The flower variant/mutant with increased in number of tepals i.e., seven instead of five was observed at 0.2% (36 hrs) in both C_1 and C_2 generation (Fig. 1I). The number and size of flowers of autotetraploid increased as compared to diploid prototypes. The length of the stamen filament was also increased in the induced autotetraploid as compared to the diploid control. The seeds of autotetraploid were much larger than diploids and the texture of the seed coat became smooth and shiny which was observed at 0.2% (36 hrs) (Fig. 1J).

Autotetraploids showed typical characteristics of

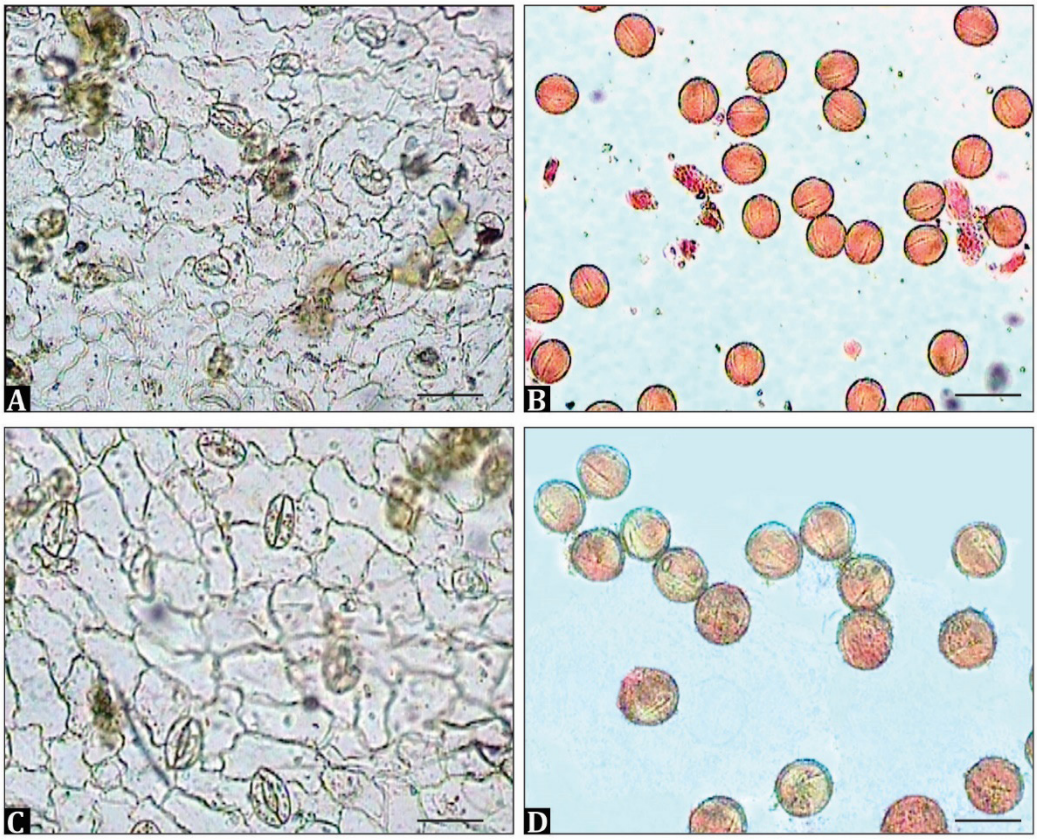

Fig. 3. Stomata and pollen study in diploid and autotetraploid plants: A = Stomata of diploid plant, B = Pollen grain of diploid plant, C = Stomata of autotetraploid plant and D = Pollen grain of autotetraploid plant

polyploidization. Initially, their growth rate was slower, it might be possible owing to lower production of growth hormone in the meristematic portions in the early stages of the plant (Kumar and Dwivedi 2017) but later they showed gigantism which is economically important for high yield production (Joshi and Verma 2004). This robustness in the morphological characters is described as gigas effects in polyploids (Sattler et al. 2016).

The rate of cell division could be reduced because of slow growth and development (Eigsti 1947) which may be the results of physiological disturbance induced by colchicine (Swanson 1957), presence of lesser growth hormone etc. (Avery and Pottorf 1945; Larsen and Mintung 1950) and due to these interruptions, the respiratory intensity is also reduced and many enzymatic activities are diminished (Stebbins 1940, 1950) and having least rate metabolic activities in autotetraploids because of delayed flowering (Biswas and Bhattacharya 1971). After some time, the polyploids attain normal growth pattern, grow vigorously and finally develop large size organ (Vainola 2000).

The study of leaf morphology is an important aspect for screening of polyploid plants at preliminary stages. The leaves of polyploids were very thick, waxy, dark green in colour, altered length and width ratio and these observations were supported by earlier findings of Conteras et al.(2009). The dark green colour of leaves of tetraploids might be due the increased number and size of chloroplast with quantitative increment in DNA content in the tetraploids (Butterfass 1983). The photosynthetic and transpiration capacities may become higher due to larger stomata and higher chlorophyll content in tetraploids as compared to diploid prototypes (Zhang et al. 2018). The increment in the flower size and number of sepals in tetraploid was very significant for improving ornamentation breeding.

Stomata observations

Initially, change in size and shape of plant species is the major characteristic of induced polyploidy (Chaudhary 1980). Therefore, stomatal size can be an indicator of ploidy level and it has been used in different plant types for estimating the ploidy levels (Hamill 1992; Chakraborti 1998; Gallon 2014). Anatomical studies emphasizing on stomata size measurements are a very amenable indicator for preliminary screening of polyploids. A significant increase in stomata size accompanied by a significant decrease in the density of the stomata was associated with an increase in ploidy level. In diploids, the mean value of length and breadth of

stomata was observed as 20.30 and 15.71 µm and stomata frequency was found to be 220 mm^{-2}. In autotetraploids, the mean value of length and breadth of stomata was observed as 42.66 µm and 33.70 µm in C_1 generation, respectively. The average of stomata frequency in colchicine induced autotetraploids was also registered which was found to be 128.00 mm^{-2} in C_1 generation (Fig. 3). The stomatal size in C_2 generation was slightly increased as compared to C_1 generation autotetraploids.

Cytological observations

The morphological observations of autotetraploid were supported by cytological study of meiosis in PMCs at microsporogenesis. In the diploid (control), the PMCs observed at diakinesis/metaphase I had 8 bivalents (2n=16) (Fig. 2A and B) and anaphase I showed normal separation of 8:8 (Fig. 2C). In the induced autotetraploids of C_1 and C_2 generations, metaphase I showed a total of 32 chromosomal complements (Fig. 2D) and 16:16 separation at Anaphase I (Fig. 2G). Figures 2E and F showed combination of bivalents, univalents and multivalents, which were observed in the following configurations $2_I+5_{II}+2_{IV}+2_{VI}$ and $8_{II}+2_{III}+1_{IV}+1_{VI}$. Besides, a number of chromosomal abnormalities were observed in autotetraploid such as precocious chromosomes at metaphase I (Fig. 2F). Autotetraploid was also reported at Metaphase II (Fig. 2H) and Fig. 2I showed unequal segregation of chromosomes at Autotetraploid metaphase II. The highest frequency observed was of type $4_I+7_{II}+2_{IV}+1_{VI}$ with the maximum being 20.00% while $8_{II}+2_{III}+1_{IV}+1_{VI}$ showed lower frequency i.e. 2.50%. These observations have been summarized in Table 3. The percentage of cytological anomalies in C_2 generation was significantly decreased as compared to C_1 generation autotetraploids. Variable changes in meiotic anomalies in the form of univalent, bivalent, tetravalent and multivalent associations have been observed in autotetraploids.

The normal process of cell division includes division of centromere and movement of chromosomes towards opposite pole but in the colchicine treated dividing cells, there is no migration of split chromosomes towards the opposite poles of the cell further no division of cell into daughter cell as it normally would. Thus, colchicine treatment results in chromosome doubling in a cell (Zielinski 1948). The genomic doubling needs a sequence of genetic and genomic alterations that regulate the proper centromere recognition, chromosome coupling and stabilized assortment of chromosome during meiosis (Yadav 2010). Multivalent formation exhibited in the autotetraploid plant during meiotic stage is typical *i.e.* association of chromosomes due to slower growth rate. Higher number of multivalents in tetraploid consequents in various other abnormalities such as laggard, bridges, irregular separation and unequal distribution of chromosomes at meiotic anaphase I/II (Darlington 1965; Griesbach and Bhat 1990) which further leads to reduction in pollen fertility. Chromosome enlargement and highest chiasmata frequency could be responsible for the formation of multivalents. The univalent is basic causal factor which is responsible for forming above mentioned abnormalities (Myers and Hill 1943).

Post-meiotic studies

Figures 2 J-L illustrated post-meiotic products such as hexad, octad and polyad which were observed at 0.4% (36 hrs.) in 13 microsporocytes. The size and no. of pollen grains of autotetraploids in C_1 and C_2 generation were observed and compared to diploid prototypes. In autotetraploids, the size of pollen grains was increased which was observed as 62.23 µm in C_1 generation and 63.46 µm in C_2 generation, respectively while the frequency of pollen grains was reduced in contrast to diploids. By using counting method in 6-8 different microscopic field (10 X resolution), the mean value of number of pollen grains was recorded as 147 and 149 in C_1 and C_2 autotetraploids whereas the control plants showed 192 pollen grains (Table 2), represented in Fig. 3. Pollen fertility was reduced in colchicine induced tetraploids. The calculated mean pollen fertility in autotetraploid and diploid pollen grains was found to be 55.63% and 92.71%, respectively (Table 2). In C_2 generation, pollen fertility was also increased as compared to C_1, which stated about the degree of meiotic stability of the autotetraploids in this generation.

Irregularities in chromosome behaviour at meiotic stage lead to reduction in pollen fertility which has also been reported by Darlington (1937) and Kostoff (1940). Hence, it is affirmed that multivalent formation at meiotic stage and pollen fertility are directly correlated with each other in this case. Thus, it is revealed that the irregularities of chromosome cannot serve as an only explanation for sterility in case of autotetraploids. Sterility may be due to involvement of same genetic and physiological factors as emphasized by Roy Tapadar (1963). Similar report on F_1 steriles was also available in *Fagopyrum* inter-specific hybridization (Woo et al. 2010). Pollen sterility gene interactions tend to increase the chromosomal abnormalities which cause the partial abortion of male gametes and leads to the decline in the seed set of the autotetraploid rice hybrids as reported by He et al. (2011).

Fertility is controlled by a system of polygenes which remains balanced in diploids and also reported in autotetraploid *Brassica* (Parthasarathy and Rajan 1953). Asynaptic condition was alsoobserved and Sjoodin (1970) suggested that this could be the result of laggards left behind during the first meiotic division. Disturbed polarity could be the result of multipolar distribution of chromatin and production of extra microspore. The sporads are highly specialized cells which are able to produce four haploid cells after a series of genetically controlled steps (Caetano-Pereira

et al. 1999). The formation of the post-meiotic products or sporads different from perfect tetrads, such as monads, dyads, triads and polyads imply the formation of gametes with more chromosomes or with eliminated chromosomes and the formation of unreduced (Golubovskaya 1989) or sterile (Bosco and Tusa 1999) pollen grains. These sporads may lead to the formation of 2n pollen grains (Kumar and Dwivedi 2013). According to Souza et al. (2003), the larger microspore may occur from two nuclei that did not segregate normally at meiosis II probably due to the irregularity in the spindle fiber orientation or even due to the absence of cytokinesis. The normal and harmonious course of meiosis during microsporogenesis would guarantee viability to the gamete, but there are post-meiotic genes, and any disturbance in such genes may break cell to cell communication, can lead to gamete malformation, making it equally not viable (Caetano-Pereira et al. 1998). Lydia and Raja Rao (1982) also analyzed the number of hexad and pentad in auto-tetraploid. The lowest fertility is one of the negative characteristics of autotetraploids because it acts as a barrier for the inheritance of polyploids but it can be rectified through further sophisticated breeding programme and selection. After subsequent studies of different aspects, the induction of polyploidy in young treated seedlings was confirmed by chromosomal counting (2n = 4x =32) through cytology which is the more efficient and convenient method as compared to the fulfillment of standard protocols of flow cytometry for the determination of ploidy levels.

The colchicine is responsible for the doubling of chromosome numbers of plant species which provide a better utilizing platform to the plant breeders. However, it cannot ensure the offering of simple magic road to the production of spectacular new horticultural varieties of genuine merit (Zielinski 1948). In the present study, autotetraploids were confirmed morphologically as well as cytologically. Polyploidy is a unique phenomenon, however, plant breeders need to be substantially acquainted on the mechanism of genomic changes as well as mode of their expression phenotypically for better exploitation of this science.

Authors' contribution

Conceptualization of research (AS, GK); Designing of the experiments (AS, GK); Contribution of experimental materials (AS, GK); Execution of field/lab experiments and data collection (AS); Analysis of data and interpretation (AS); Preparation of the manuscript (AS, GK).

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References

Adams K.L. and Wendel J.F. 2005. Polyploidy and genome evolution in plants. Curr. Opin. Plant Biol., **8**: 135–141.

Avery G. S. and Pottorf L. 1945. Polyploidy, auxin and nitrogen in green plants. Am. J. Bot., **32**: 669-671.

Bishwas A. K. and Bhattacharyya N. K. 1971. Induced polyploidy in Legumes I.*Cyamopsispsoraloides DC*. Cytologia, **36**: 469-479.

Blakeslee A. F. and Avery A. G. 1937. Methods of inducing doubling of chromosomes by treatment with colchicine. J. Hered., **28**: 392-411.

Bosco S.F., Tusa N. andConicella C. 1999. Microsporogenesis in a citrus interespecific tetraploid somatic hybrid and its fusion parents. Heredity, **83**: 373-377.

Butterfass T. 1983. Anucleotypic control of chloroplast reproduction. Protoplasma, **118**: 71-74.

Caetano-Pereira C.M., Defani-Scoarize M.A., Pagliarini M.S. and Brasil E.M. 1998. Syncytes, abnormal cytokinesis and spindle irregularities in maize microsporogenesis. Maydica, **43**: 235-242.

Caetano-Pereira C.M., Pagliarini M. S. and Brasil E.M. 1999. Cell fusion and chromatin degeneration in an inbred line of maize. Genet. Mol. Biol., **22**. São Paulo.

Cawoy V., Ledent J. F., Kinet J. M. and Jacquemart A. L. 2009. Floral Biology of Common Buckwheat (*Fagopyrum esculentum* Moench). Eur. J. Plant Sci. Biotechnol., **3**: 1-9.

Chakraborti S. P., Vijayan K., RoyB. N. and Qadri S. M. H. 1998. In vitro induction of tetraploidy in mulberry (*Morus alba* L.). Plant Cell Rep., **17**: 799-803.

Chaudhari H. K. 1980. Elementary principles of plant breeding. Oxford and IBH publishing Co. Pvt. Ltd. New Delhi. pp.155-191.

Chrungoo Nikhil K. and Chettry U. 2021. Buckwheat: A critical approach towards assessment of its potential as a super crop Indian J. Genet. Plant Breed., **81**(1): 1-23. DOI: 10.31742/IJGPB.81.1.1

Comai L. 2005. The advantages and disadvantages of being polyploid. Nat. Rev. Genet., **6**: 836–846.

Contreras R.N. and Ruter J.M. 2009. An oryzalin-induced autoallooctoploid of *Hibiscus acetosella* 'Panama Red'. J. Amer. Soc. Hort. Sci., **134**: 553–559.

Darlington C. D. 1937. Recent Advances in Cytology. J. and A. Churchill. London.

Darlington C.D. 1965. Recent advances in Cytology. J. and A. Churchill Ltd. London.

Eigsti O. J. 1947. The pollen tube method for making comparisons of differences in meiotic rates between diploid and tetraploid. Genetics, **32**: 85.

Gallone A., Hunter A. and Douglas G. C. 2014.Polyploid induction in vitro using colchicine and oryzalin on Hebe 'Oratia Beauty': Production and characterization of the vegetative traits. Sci. Hortic., **179**: 59-66.

Golubovskaya I.N. 1989. Meiosis in maize: *mei*genes and conception of genetic control of meiosis. Advances in Genetics, **26**: 149-192.

Griesbach R. J. and Bhat R. N. 1990. Colchicine-induced polyploidy

in *Eustoma grandiflorum*. Hort. Sci., **25**: 1284-1286.
Kreft I., Zhou M., Golobm A., Germm M., Likarm M., Dziedzicm K. and Luthar Z. 2020. Breeding buckwheat for nutritional quality. Breed. Sci., **70**: 67-73.
Liu G., Li Z. and Bao M. 2007. Colchicine induced chromosome doubling in *Platanus acerifolia* and its effect on plant morphology. Euphytica, **157**: 145-154.
Hamill S. D., Smith M. K. and Dodd W. A. 1992. In vitro induction of banana autotetraploids by colchicine treatment of micropropagated diploids. Aust. J. Bot., **40**: 887–896.
He J. H., Shahid M. Q., Li Y. J., Guo H. B., Cheng X. A., Liu X. D. and Lu Y. G. 2011. Allelic interaction of F1 pollen sterility loci and abnormal chromosome behaviour caused pollen sterility in inter-sub-specific autotetraploid rice hybrids. J. Exp. Bot., **62**: 4433-4445.
Joshi P. and Verma R. C. 2004. High frequency production of colchicine induced autotetraploids in Faba Bean (*Vicia faba* L.). Cytologia, **69**: 141-147.
Kotstoff D. 1940. Fertility and chromosome length correlation between chromosome length and viability of gametes in autotetraploid plants. J. Hered., **31**: 33-34.
Kumar G and Dwivedi K. 2013. Cytogenetical evidences of abnormal meiosis and 2n pollen formation via colchicine in microsporogenesis of *Brassica campestris* L. Inter. J. Res. Plant Sci., **3**: 18-24.
Kumar G. and Dwivedi K. 2014. Induced polyploidization in *Brassica campestris* L. (Brassicaceae). Cytol. Genet., **48**: 103–110.
Kumar G. and Dwivedi H. 2017. Induced Autotetraploidy in *Trachyspermum ammi* (L.) Sprague (Apiaceae). Cytology Genet., **51**: 391–400.
Kushwah K.S., Verma R.C., PatelS. and Jain N.K. 2018. Colchicine induced polyploidy in *Chrysanthemum carinatum* L. J. Phylo. Evol. Biol., **6**: 1.
Larsen P. and Mintung S. 1950. Growth promoting and growth retarding substances in pollen from 2n and 3n apple varieties. Bot. Gaz., **3**: 437-447.
Love A. and Love D. 1949. The geobotanical significance of polyploidy. Portugaliae Acta (Suppl), 273–352.
Lydia G. and Raja Rao K. G. 1982. Abnormal spindle behaviour in induced autotetraploid, *Physalis pubescens* L. Theor. Appl. Genet., **63**: 125-127.
Müntzing, A. 1936. The evolutionary significance of autopolyploidy. Hereditas, **21**: 263-378.
Myers W. M. and Hill H. D. 1943. Increased meiotic irregularities accompanying breeding of *Dactylisglomerata*. Genetics, **28**: 383-397.
Omidbaigi R., Mirzaee M., Hassani M. and Moghadam M. 2010. Induction and identification of polyploidy in basil (*Ocimum basilicum* L.) medicinal plant by colchicine treatment. Int. J. Plant Prod., **4**: 87–98.
Osborn T.C., Pires J.C., Birchler J.A., Auger D.L., Chen Z.J., Lee H.S., Comai L., Madlung A., Doerge R.W., Colot V. and Martienssen R.A. 2003. Understanding mechanisms of novel gene expression in polyploids. Trends Genet., **19**: 141-147.
Panda D., Goode B.L., Feinstein S.C. and Wilson L. 1995. Kinetic stabilization of microtubule dynamics at steady state by tau and microtubule-binding domains of tau. Biochem., **34**: 11117–11127.
Parthasarathy N. and Ranjan S. S. 1953. Studies in the fertility of autotetraploids of *Brassica campestris* var. toria. Euphytica, **2**: 25-30.
Ramsey J. and Schemske D.W. 1998. Pathways, mechanisms, and rates of polyploid formation in flowering plants. Annu. Rev. Ecol. Syst., **29**: 467–501.
Renny-Byfield S. and Wendel J.F. 2014. Doubling down on genomes: polyploidy and crop plants. Am. J. Bot., **101**: 115.
Roy Tapadar N. N. 1963. Studies in induced tetraploids of the family Apocynaceae I. *Rauvolfia serpentine* Benth. Cytologia, **28**: 229-241.
Sattler M.C., Carvalho C.R. and Clarindo W.R. 2016. The polyploidy and its key role in plant breeding. Planta, **243**: 281–296.
Schubert I., Rieger R. and Kunzel G. 1991. Karyotype reconstruction in plants with special emphasis on *Vicia faba* L. In: Gupta P. K. and Ysuchiya T. (eds.). Chromosome Engineering in Plants: Genetics, Breeding, Evolution, Part A. Amsterdam Elsevier Science Publishers. pp. 113-140.
Sjödin J. 1970. Induced asynaptic mutants in *Vicia faba* L. Hereditas, **66**: 215-232.
Soltis D.E., Albert V.A., Leebens-Mack J., BellC.D., Paterson A.H., Zheng C., Sankoff D., Depamphilis C.W., Wall P.K. and Soltis P.S. 2009. Polyploidy and angiosperm diversification. Am. J. Bot., **96**: 336–348.
Souza M.M., Pereira T.N.S., Viana A.P., Pereira M., Bernacci L.C., Sudré C.P. and Silva L.C. 2003. Meiotic irregularities and pollen viability in *Passiflora edmundoi* Sacco (Passifloraceae). Caryologia, **56**: 161-169.
Stebbins G.L. 1940. The significance of polyploidy in plant Evolution. Am. Nat., **74**: 54–66.
Swanson C. P. 1957. Cytology and Cytogenetics. Prentice-Hall Inc. Johns Hopkins Univ., Baltimore.
Talukdar D. 2010. Cytogenetic characterization of induced autotetraploid in grass pea (*Lathyrus sativus* L.). Caryologia, **63**: 62–72.
Tian X., Ruirui L.iu R., Tian B. and Liu J. 2009. Karyological studies of Parapteropyrum and Atraphaxis (Polygonaceae). Caryologia, **62**(4): 261-266.
Vainola A. 2000. Polyploidization and early screening of *Rhododendron* hybrids. Euphytica, **112**: 239–244.
Van de Peer Y., Maere S. and Meyer A. 2009. The evolutionary significance of ancient genome duplications. Nat. Rev. Genet., **10**: 725–732.
Woo, Sun-Hee and Kamal, Abu Hena and Tatsuro, Suzuki and Campbell, Clayton and Adachi, Taiji and Yun, Young-Ho and Chung, Keun-Yook and Choi and Jong-Soon. 2010. Buckwheat (*Fagopyrum esculentum* Moench.): Concepts, Prospects and Potential. The European J. Plant Sci. Biotechnol., **4**: 1-16.
Yadav R. S. 2010. Impact of genome doubling on cytomorphological characters of *Sesamum indicum* L. (Pedaliaceae). Chromosome Bot., **5**: 43-47.
Zhang H., An S., Hu J., Lin Z., Liu X., Bao H. and Chen, R. 2018. Induction, identification and characterization of polyploidy in *Stevia rebaudiana* Bertoni. Plant Biotechnol., **35**: 81–86.
Zhou Y., Jiang S., Lu R., Gu Y. and Ren Q. 1993. Research on the breeding of *Platanus occidenalis* Linn. without cone. Acta. Hort. Sin., **20**: 295-298.
Zielinski Q. 1948. The use of Colchicine in Plant breeding. Agricultural Experiment Station, Oregon State College Win. A. Schoenfeld, Director Corvallis. Circular of Information No. 420.