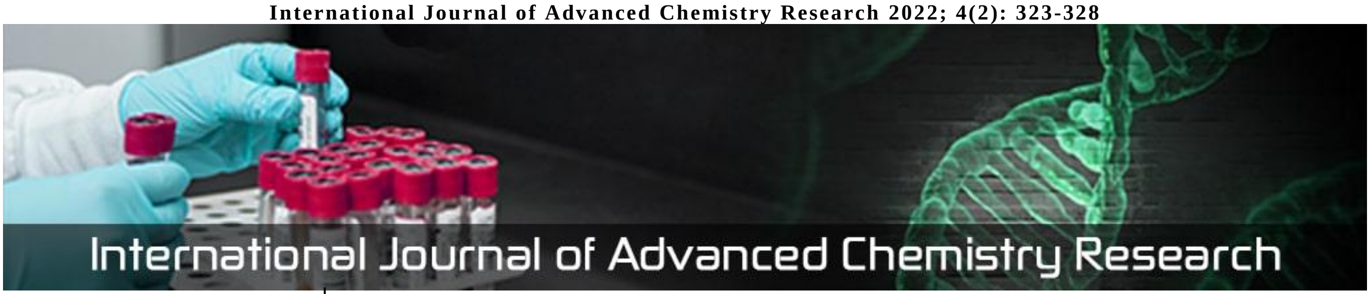
International Journal of Advanced Chemistry Research 2022; 4(2): 323-328



ISSN Print: 2664-6781
ISSN Online: 2664-679X
Impact Factor: RJIF 5.32
IJACR 2022; 4(2): 323-328
www.chemistryjournals.net
Received: 19-07-2022
Accepted: 21-09-2022

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Induced, identify and study the impact colchicine treated *Lathyrus sativus* L. (Var. Prateek)

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Abstract
India's third-most significant dual purpose cold season pulse crop, lathyrus, covers 0.58 million acres and produces 0.43 million tonnes annually. Increasing vegetative growth might be a practical method of increasing feed output. Inducing auto-polyploidy and comparing the polyploids to their diploid counterparts are the two main objectives of this investigation, to find out whether polyploidy has any advantages over its diploid counterparts. Three different colchicine concentrations were used to treat the seeds of the Prateek genotypes of grass pea (*Lathyrus sativus* L.) to induce polyploidy (0.02 percent, 0.04 percent and 0.05 percent). Their diploid counterparts were compared to see how polyploidy differ them. Observations of stomata, nucleus size, plant height, number of branches per plant, leaf area, and number of leaves are all morphological attributes that are more varied. There are considerable variations in flower and seed proportions. The amount of seeds and stomatal cells were significantly different in a negative way. Size of the nucleus (µm2), stomatal length and width(µm), blossom length (cm) and width (cm), days to first blooming, number of flowers per plant, number of seeds per plant, seed length (mm), seed width (mm) and ten seed weight were all found to have positive significant differences. Induction of polyploidy produced a taller, more branching plant with an increase in seed weight of 10 seeds. This strategy could be used in plant breeding initiatives to increase the yield of fodder.

Keywords: Lathyrus, colchicine, polyploidy

Introduction
Lathyrus or Grasspea is the third largest cold season legume crop in India, covering an area of 5, 80,000 hectares and an annual production of 4,30,000 tonnes. It is mainly grown in Bihar, Madhya Pradesh, Maharashtra, West Bengal and Chhattisgarh. Most of the area (about 70%) is shared by Chhattisgarh and Vidarba in Maharashtra (Dixit *et al*., 2016) [5].

Lathyrus or Grass pea has a great scope as food, feed and fodder crop under climate change. Consumption of grass pea as a normal diet is safe (Singh and Rao, 2013) [15] and holds promise in diversified agri-food systems in rainfed dry land regions. The worldwide demand for high-value animal protein and grass pea could be a potential source of protein for animal feeds. Lathyrus is a valuable crop since it serves as both fodder and feed. Because of its minimal input nature, developing a grass pea variety for fodder is a superior choice, either through traditional breeding or through unique breeding techniques (Mutation, Polyploidy etc.).

According to Tavan *et al*. (2015) [16], polyploidy is the situation of having more than two sets of chromosomes and is also a key process of plant evolution. Both wild and domesticated plants have evolved in large part as a result of polyploidy. In some circumstances, polyploid organisms surpass their diploid relatives in various ways and frequently show enhanced vigour. Induced polyploid leads to crop’s better vigour, higher adaptability to environmental conditions, increased cell size which is the prerequisite to fodder crops. Other benefits of being polyploid include better salinity, drought, or extreme temperature stress tolerance (Sattler *et al*., 2016) [13].

The purpose of induce polyploidy, evaluate and to assess phenotypic and genome size variations in putative autopolyploids of Grasspea or Lathyrus in relation to their diploid counterparts derived from genetically homogenous plant material treated with colchicine.

Material and Methods

Seeds of *Lathyrus sativus* L. (Variety - Prateek) were treated with the colchicine, in order to induce polyploidy. Seeds were soaked in colchicines solution of 0.02%, 0.04% and 0.05% for 2.5 hours in falcon tube. 100 seeds were taken for each treatment. Meanwhile, control seeds were also soaked in falcon tube containing distilled water. After specific period of the treatments seeds were carefully rinsed with distilled water. Furthermore, treated and control seeds were placed to protrays containing cocopeat as a germinating/growth substratum for sixteen days under comparable conditions, Sixteen days after sowing, these seedlings were transfer to field.

Leaf area were measured by using Image J software for leaf analysis. Increased leaf dimensions were recorded and the averages were taken. Leaf analysis was done using following steps: From the desktop, opened the Image j software. Then Open leaf image via Select File→ Open Samples→ Leaf, Convert scanned color image of leaf to grayscale then Set measurement scale, Threshold the leaf image using the automated routine, Threshold new image of leaf using manual settings, Calculate area of entire leaf.

Stomata were studied by peeling off the epidermal layer of leaf. Take the leaf and peel from the lower surface of the leaf using the forceps. Then cut a small piece and take the microscopic slide and place it add 2-3 drops of acetone-carmine stain, after this crushed the epidermal layer with the help of needle. Then put a coverslip and observed under Trinocular microscope with digital display system optima camera type-DG1510CCD and DE winter software was used. Images were taken at 100 X magnification (10 X objective × 10 X eyepiece).

For nucleus studies 1-1.5 cm. root tips were fixed between 8-10 am in carney's solution (6:3:1) (Ethanol: Glacial acetic acid: Chloroform) for 24 hours and preserved in 70% alcohol for future studies. After washing the fixed root tips in distilled water put it in a 1.5 µl eppendorf tube containing 1N HCl and hydrolyzed for 10 minutes at 60 ℃. After that, took the roots and kept in Schiff's reagent, the vials were covered with aluminium foil and left in the dark for 30 minutes. The samples were put on a clean microscopic slide cutted a very fine root segment add 2-3 drops of acetone-carmine stain, After this crush the root tips with the help of needle. Then put a coverslip and gently squshed the roots. Observed under Trinocular microscope with digital display system optima camera type- DG1510CCD and Dewinter software was used. Images were taken at 100 X magnification (10 X objective × 10 X eyepiece).

Results

Variety of grass pea namely, Prateek was taken for induction of polyploidy by seed treatment method at different colchicine concentration where germination occur after 1-2 days. Thereafter, completing one week in cocopeat, germination percentage was estimated. Sixteen days after sowing, these seedlings were transferred to field for their morphological observations. The impact of colchicine treatment on germination and survival percentage after sowing and transplanting the germinated seedlings to protray and field, respectively was also assessed in Figure 1.

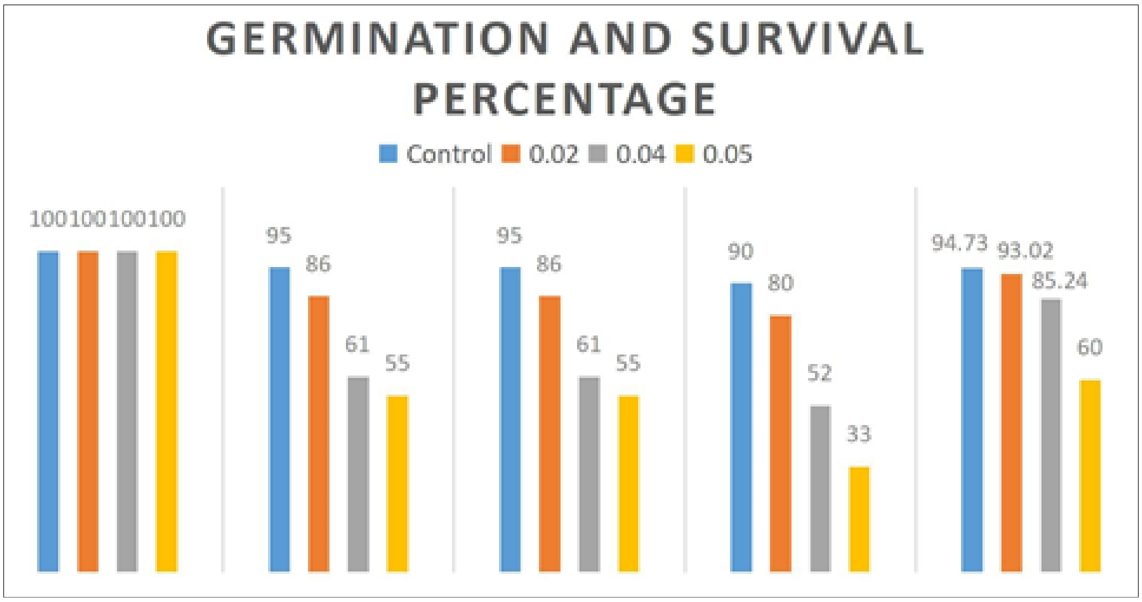

Fig 1: Germination & survival percentage after treatment

Morphological parameters (Plant height, number of branches per plant, number of leaves per plant, leaf length, leaf width and leaf area) were observed in the polyploid-induced genotypes when compared to control. The leaves in putative tetraploids were broader, longer and thicker as compare to diploids plants.

Putative tetraploid plants had larger epidermal cell and stomata as compare to diploids, but number of stomata per unit area were much less in the putative tetraploid plants. In general, polyploids have larger stomata at lower density compared to those of diploids, which is one of the hallmark characteristics of polyploidy. The same result was concluded by Leal-Bertioli *et al.*, 2012.

Table 1: Morphological features of diploid and putative tetraploids plants of Lathyrus sativus L. (Var. Prateek)

Character	Diploids		Putative tetraploids		t-test value
	Range	Mean	Range	Mean	
Plant height(cm)	28.4-43.1	44.34	32.9-59.4	55.42	7.67
Number of branches per plant	0-4	4.08	1-5	7.26	2.13
Number of leaves per plant	64-168	60.93	69-184	66.26	1.88
Leaf length (cm)	4.3-5.5	5.59	4.5-6.0	5.63	3.40*
Leaf width (cm)	0.5-0.7	0.87	0.5-0.9	0.98	6.23*
Leaf area (m2)	9.4-9.69	10.68	10.68-1.09	11.61	23.12*
Days to first flowering	38-43	40.05	48-55	51.66	7.71*
Number of flowers per plant	18-24	21.00	24-35	27.16	4.17*
Flower length (cm)	1.2-1.5	1.31	1.9-2.2	2.03	5.26*
Flower width (cm)	0.4-1.0	0.55	0.8-1.1	0.95	4.08*
Number of pods per plant	10-12	10.66	13-16	14.05	7.53*
Seed length(mm	44-53	48.03	52-60	55.06	5.84*
Seed width (mm)	4.5-5.1	4.09	5.5-5.7	5.05	4.30*
10 seeds weight (g.)	0.48-1.06	0.07	1.05-1.21	1.01	4.81*
Nucleus	1302.56-	1651.90	1422.22-	1725.02	2.33*
size(μm2)	1816.57		1915.24		
Stomata	105.66-	114.72	109.26-	123.46	12.46*
Length ((μm)	129.73		134.84		
Stomata Width (μm)	44.43-55.28	48.27	60.25-66.56	63.38	11.57*
Stomata number	8-12	10.5	4-6	5.37	-12.04*

*Significant at 0.05 probability level

A preliminary screening for putative autotetraploids was conducted based on morphological characteristics, Plant height(cm), Number of branches per plant, Number of leaves per plant, Leaf length and width, Days of first flowering, Flower length and width, Number of pods per plant, Seed length and width and 10 seed weight.

The plant showing differences in stomata length, stomata width and less number of stomata were selected as putative autotetraploids (Fig 2).

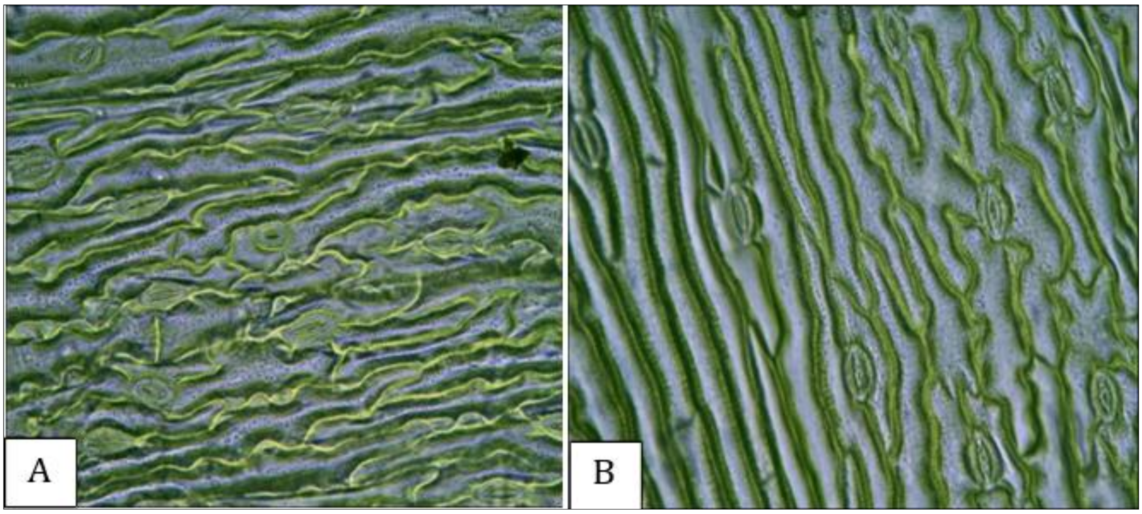

Fig 2: Number of stomata diploid (A) and Putative tetraploid (B)

In their experiment, Cavalier-Smith (2005) [3] and Jovtchev *et al*. (2006) [8] found that species with larger genomes typically have larger nuclear and cellular volumes,

Supporting the hypothesis that "DNA may affect nuclear volume, because the size of the nucleus could be directly proportional to the amount of DNA it contains and the extent to which that DNA is compacted." According to him, the total amount of DNA can be used to predict nuclear volume by an evident mechanism that involves basic space filling and attachment to the nuclear matrix and envelope, both of which have plenty of supporting evidence (Fig 3).

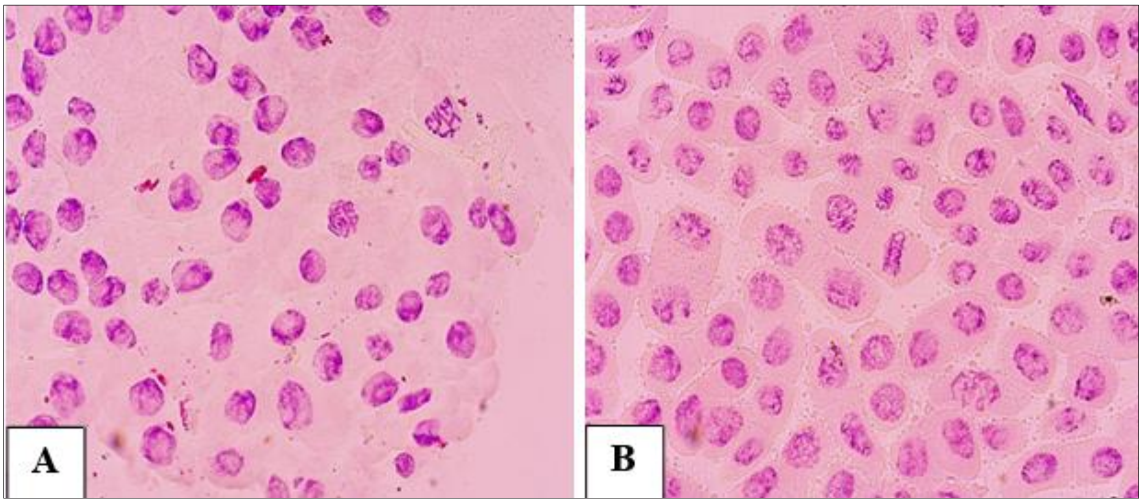

Fig 3: Variations between size of nucleus diploid (A) and putative tetraploid (B)

Liqin *et al*., 2019 [10] stated "There are numerous positive effects of induced polyploidy when compared with diploidy in various forage species; Hence, concluded that putative Colchiploids have larger leaf area when compared with diploids. Different sizes of leaves at different concentrations were also observed (Fig 4).

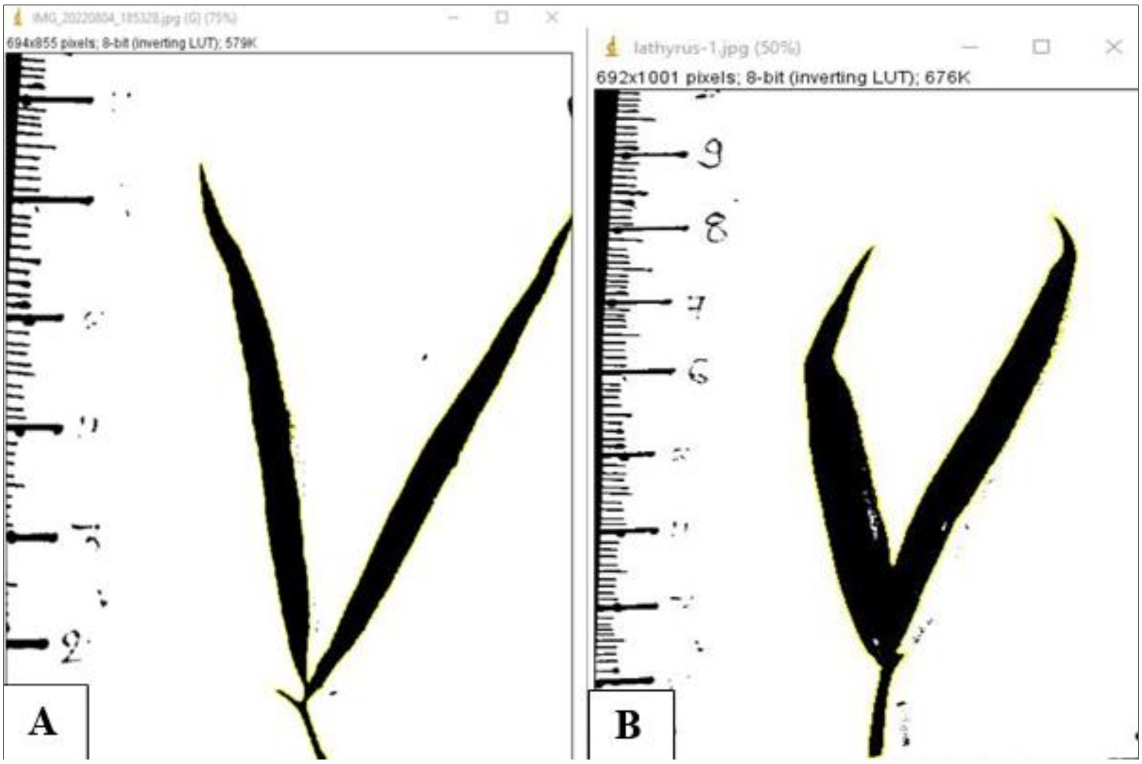

Fig 4: Variations between areas of leaf diploid (A) and putative tetraploid (B)

Seed dimensions of diploids and induced tetraploids were examined. Large size seeds were observed in putative colchiploids than diploids (Fig 5). Similar results were also reported by (Maceira *et al*., 1993; Bretagnolle *et al*., 1995; Hoya *et al*., 2007; Cohen *et al*., 2013) [11, 2, 7, 4].

Fig 5: Variations between size of seeds diploid (A) and putative tetraploid (B)

Discussion

According to Elgsti and Dustin (1955) [6], polyploidy can be distinguished by delayed plant growth, thicker, darker-green leaves, larger stomata and pollen grains, high pollen sterility, larger flowers and seeds and slower plant growth. In this study, phenotypic differences between diploid and colchiploids grass pea plants were identified through seed treatment. These differences included plant height, branch and leaf number and size, days to first blooming, flower number and size, and number of pods per plant. Putative tetraploids had the largest nuclei and the least stomata, according to cytological studies, although their seed weight (g) was higher than that of diploids, size of the individual cells as well as.

Total six putative polyploid obtained, out of which two in 0.02%, three in 0.04% and one in 0.05%. The induction of autotetraploids in the current study can also account for the increased height of the plants, increased tillering and branching per plant, significant increases in leaf dimensions and leaf area, flower dimensions, the number of flowers per plant, delayed flowering, negative significant differences for the number of seeds per plant and increased seed weight. A positive substantial rise in nucleus size was seen and this could be attributed to an increase in ploidy level. Stomatal and nucleus size were observed to be increasing positively, which is consistent with the plant's increased ploidy level And decreasing negatively. Similar findings in Vicia faba L. were confirmed by Ardabili *et al*. (2015) [1], Shreshtha and Kang (2016) [14] and Nagat *et al*. (2020) [12].

Conclusion

The second treatment (0.04 percent), which was used to generate polyploidy, produced the most putative autopolyploids out of the three treatments used: 0.02 percent, 0.04 percent and 0.05 percent. Additionally, 0.04 percent exhibited a little bit more variations in verified tetraploids than the other two treatments. In terms of plant height and leaf area, autotetraploids produced more fodder than diploids. Induced tetraploids had more branches, more leaves, more flowers, delayed flowering, bigger leaves, bigger flowers, more pods, bigger seeds and heavier seeds When compared to their diploid counterparts. Both the number of stomata and number of seeds per plant reduced.

The results suggest that this polyploidy technology might be applied to the breeding of fodder. Genetically polyploidy Plants contained several alleles, which may help to enhance allelic variety and provide numerous evolutionary and adaptive benefits. In some polyploid plants, no seed setting were also seen.

Acknowledgement

The authors are thankful to the Department of Plant Molecular Biology and Biotechnology and Richharia Research Lab (RRL), College of Agriculture, IGKV, Raipur for the polyploidy work and lab facilities.

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