

## Interbivalent connections and pollen fertility in *Lupinus polyphyllus* Lindl. (Fabaceae)

Syed Mudassir Jeelani<sup>1</sup>, Younas Rasheed Tantray<sup>2</sup>,

Reyaz Ahmad Malik<sup>3</sup>

<sup>1</sup>Indian Institute of Integrative Medicine, Jammu – 180001. (India)

<sup>2,3</sup>Department of Botany, Punjabi University Patiala -147002. Punjab (India)

### ABSTRACT

Cytomorphological study was carried out on three populations of *Lupinus polyphyllus* varying in flower colour from Kashmir Himalaya. All the three populations depicted same chromosome number of  $2n = 48$  at different stages of meiosis. The meiotic course in all the three populations is unusual showing interbivalent connections at different meiotic stages in large number of PMCs. Further, some variation among the size of fertile pollen grains along with pollen sterility was recorded.

**Key Words:** *Lupinus polyphyllus*, Interbivalent connections, Kashmir Himalaya, Pollen fertility.

### I. INTRODUCTION

The topography and climate have immense effect on the vegetation patterns of Kashmir Himalayas, exposing the plants to diverse habitats. All this results into considerable amount of genetic diversity in the form of various meiotic irregularities as well as huge variation in morphology.

*Lupinus L.* is one of the larger genera of family Fabaceae and geographically widespread, comprising of 275 species of annual herbs as well as herbaceous and woody perennials. The majority of species occur in the New World, with two main centers of diversity in Western North America and Andes [1]. *L. polyphyllus* Lindl. is categorized in the list of alien flora of Kashmir [2]. Although commonly cultivated as a garden plant, but also grows wild in various parts of Kashmir. The species is found on open and sunny habitats and grow on poor acidic soils. It is characterized as perennial herb having leaflets 1-9 and seeds less than 5 mm, shining with variable flower colours.

The interbivalent connections were first time reported in the polyploid plants by Kuwada [3] and since then have been reported in many plants [4, 5, 6, 7, 8, 9]. The basic information regarding the cytological status, meiotic behaviour and pollen fertility is not available for the species growing in the Kashmir Himalaya even from India. Previously, the cytological studies on the species are available from outside India only. Further, the data and information obtained from such cytomorphological surveys would be of huge significance and certainly provide a helping hand in understanding the cytological evolution of the species growing in the Kashmir Himalaya. Therefore, the present study has been carried out to reveal the chromosome number, meiotic course and effect of interbivalent connections on pollen fertility in the species.

## **II. MATERIAL AND METHODS**

During present investigations, materials for cytomorphological studies were collected from the wild plants growing in Gulmarg (34°03' N 74°23' E) of Kashmir Himalaya. For meiotic studies, the young flower buds of appropriate size were collected during the peak flowering period. These buds were fixed in Carnoy's fixative (6 parts ethyl alcohol, 3 parts chloroform and 1 part glacial acetic acid) for 24-48 hours. Then the material was transferred to 75% ethyl alcohol and stored in a refrigerator at about 4-10°C. For chromosomal preparations, smears of the young anthers were made in 1% acetocarmine. Pollen fertility was determined through the stainability of pollen grains in 50% glyceracetocarmine. Only well-filled pollen grains and those with well-stained nuclei were apparently taken as fertile and viable. The pollen grain size was measured with ocular micrometer. Photomicrographs of PMCs, for chromosomal counts, meiotic irregularities, sporads and pollen grains were made from the freshly prepared slides using a digital imaging system of *Leica Qwin*, ver. V 2.3 (Leica Microsystems, United Kingdom). Voucher specimens were deposited in the Herbarium, Department of Botany, Punjabi University, Patiala (PUN).

## **III. RESULTS**

Presently, three accessions of *L. polyphyllus* have been worked out cytomorphologically from Gulmarg in Kashmir Himalaya. The three accessions show variation in the flower colour, i.e., blue, pink and white (Figs. A-C). All the three accessions existed on same chromosome number of  $2n = 48$  at different stages of meiosis (Figs. D-F). The meiotic course in all the three accessions is abnormal which invariably show interbivalent connections at different meiotic stages in large number of PMCs (Figs. G-L). However, such connections have been observed to be more prominent during diakinesis and metaphase-I and less at other stages. The number of bivalents per group involved in such connections ranges from 2-5. Further, some variation among the size of fertile pollen grains along with pollen sterility has been recorded in all these accessions (Figs. M-O; Table 1).

## **IV. DISCUSSION**

The species is cytologically worked out for the first time from Indian and the chromosome count of  $2n = 48$  confirms the previous reports from outside India [10, 11, 12]. Earlier, the genus was considered to be of polyploid origin, having high chromosome numbers and interspecific variation [13]. Over all 118 species/ 143 cytotypes are known including 3 species from India strictly with  $2n = 48$ . At world level, 107 species (90.67%) show polyploidy with highest level at  $8x$  and intraspecific euploidy based on  $x = 12$  in 6 species. From India, all the 3 species are polyploids. The genus shows basic chromosome numbers as  $x = 7, 9, 12, 16, 17, 19, 20, 21, 22, 25, 26$  with  $x = 12$  being the most common.

According to Viinikka & Nokkala [14] the fusion of heterochromatic regions of some of the chromosomes together early in the first meiotic division probably results in the formation of chromatic knots, which lead to the formation of prominent interconnections and can be observed at various stages of meiosis. They are also of opinion that the main function of the interconnections is to keep the bivalents in contact to each other before the spindle fibers are formed, in order to guarantee the proper movements and orientation of the chromosomes in division plane. Cytomixis is generally considered responsible for such connections. However, no cytomixis or any other meiotic abnormality has been observed in the individuals of *Lupinus polyphyllus*. According to Thomas &

Revell [15], chromosomal stickiness is also responsible for such associations and applies well to the presently investigated individuals of the species. The interbivalent connections have also been held responsible for the occurrence of secondary associations in *Cicer arietinum* [15] and *Alysicarpus* [16]. However, no secondary associations have been observed.

The variation among the flower colour of the individuals of the species growing in the same environmental and climatic factors is proposed to be under genetic control.

## REFERENCE

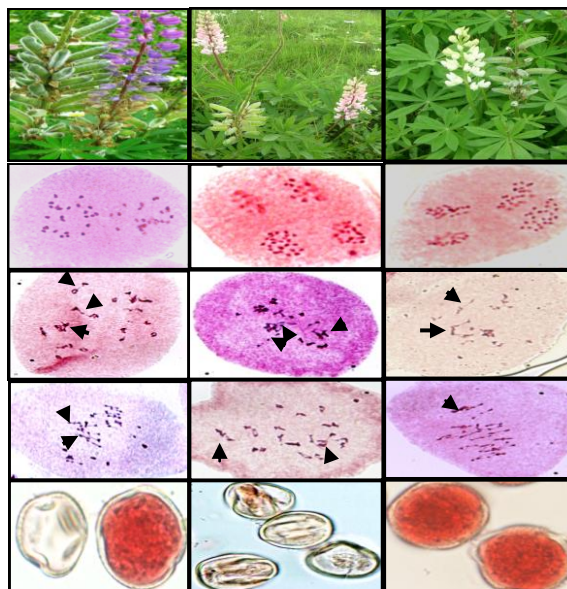
- [1] CE Hughes and RJ. Eastwood, Island radiation on a continental scale: exceptional rates of species diversification after uplift of the Andes. Proc. Nat. Acad. Sci., USA 103, 2006, 10334-10339.
- [2] AA Khuroo, I. Rashid, Z. Reshi, GH. Dar, and BA. Wafai, Alien flora of Kashmir Himalaya, Biological Invasions, 9, 2007, 269-292.
- [3] Y Kuwada, A cytological study of *Oryza sativa* L. Botanical Magazine Tokyo, 24, 1910, 267-280.
- [4] KE Akpabio, Chromosomal interconnections and metaphase-I clumping in meiosis of four species of *Crotalaria* L. Nig. J. Bot. 3, 1990, 191-195.
- [5] OA Falusi, Interchromosomal connections and metaphase-1 clumping in meiosis of two *Capsicum* L. species in Nigeria. Afr. J. Biotech., 5, 2006, 2066-2068.
- [6] VK Singhal and P. Kumar, Impact of cytomixis on meiosis, pollen viability and pollen size in wild populations of Himalayan poppy (*Meconopsis aculeata* Royle). Journal of Biosciences, 33, 2008, 371-380.
- [7] P Kumar, VK. Singhal, D. Kaur, and S. Kaur, Cytomixis and associated meiotic abnormalities affecting pollen fertility in *Clematis orientalis*. Biologia Plantarum, 54, 2010, 181-184.
- [8] R Massoud, R. Karamian, and S. Nouri, Impact of cytomixis on meiosis in *Astragalus cyclophyllus* Beck (Fabaceae) from Iran. Caryologia, 64, 2011, 256-264.
- [9] RA Malik, and RC. Gupta, Meiotic studies in some selected members of Gamopetalae from Kashmir Himalaya, Plant Systematics and Evolution, 299, 2013, 1549-1560.
- [10] E Pogan, H. Sawicka, and A. Jankun, Further studies in chromosome numbers of Polish Angiosperms, part 22. Acta Biol. Cracov. Ser. Bot., 31, 1990, 1-17.
- [11] A Murín, Karyologické atúdium okrasn@3ych rastlín flóry Slovenska. Biologia (Bratislava) 48, 1993, 441-445.
- [12] LV Semerenko, Chromosome numbers in some members of the families Asteraceae, Fabaceae, Orchidaceae and Poaceae from the Berezinsky Biosphere Reservation (Byelorussia) flora. Bot. Zhurn. (Moscow & Leningrad) 74, 1989, 1671-1673.
- [13] F Kong, E. Rudloff, RJ. Snowdon, and YP. Wang, Chromosomal distribution of 18S-25S rDNA in four *Lupinus* species visualized by fluorescence in situ hybridization. Genetika, 45, 2009, 1148-1152.
- [14] Y Viinikka, and S. Nokkala, Interchromosomal connections in meiosis of *Secale cereale*. Hereditas, 95, 1981, 219-224.

[15] PT Thomas, and SH. Revell, Secondary association and heterochromatic attraction. I. *Cicer arietinum*.  
Ann. Bot., 38, 1946, 159-164.

[16] M Sanjappa and RP. Bhatt, Cytology of the genus *Alysicarpus* (Fabaceae). Cytologia, 43, 1978, 35-44.

**Table:** Information on Accession number (PUN), Locality, altitude, present chromosome number, ploidy level, nature of meiotic course, pollen size and flower colour in different accessions of *Lupinus polyphyllus* Lindl.

Accession No.	Locality with altitude (m)	Chromosome number (2n)	Ploidy level / meiotic course	Pollen fertility (%)	Pollen size (µm)	Flower colour
54826	Gulmarg 2,600	48	4x/ A	79.02	32.81×32.73- 30.99×30.87	Bluish
56212	Gulmarg 2,600	48	4x/ A	78.89	32.90×32.75- 30.94×30.82	Pinkish
56213	Gulmarg 2,600	48	4x/ A	73.00	32.55×32.61- 30.72×30.66	Whitish



**Figure1.** Male meiosis in *Lupinus polyphyllus*:  
(A) Bluish Population; (B) Pinkish