



**STERILITY IN ALLIUM ROYLEI STEARN –  
A LESSER EXPLORED TAXON**

**BEETIKA KOHLI\* AND VEENU KAUL**

*Department of Botany, University of Jammu, Jammu 180006*

**ABSTRACT**

Plants of *Allium roylei* Stearn, a threatened taxon of North – West Himalayas studied are complex translocation heterozygotes. Structural alterations imposed thereby on the chromosomes adversely affect the reproductive features of the species. Pollen grains formed share variable number of chromosomes ranging from 1 to 8 instead of the normal. This drastically acts down their siring potential by rendering them non – viable. Despite being a prolific flower producer, the fruits and seeds set are negligible. Whatever little seed is formed, it does not germinate properly nor does it transform into a healthy plant.

**KEYWORDS :** *Allium roylei*, Sterility, Translocations, *Oenothera*, Multivalents.



**BEETIKA KOHIL**

Department of Botany, University of Jammu, Jammu 180006, India

## INTRODUCTION

Sterility in flowering plants can be expressed by the arrest in the differentiation of sex organs to different degrees and/or production of varying proportions of abortive pollen grains and/or ovules or both. Being of common occurrence in plants and drastically affecting a plant's very survival, sterility has received considerable attention. Expressed by the failure of anthers to dehisce or the pollen grains to attain maturity prior to dehiscence, sterility can also be imposed on account of abnormal development of megaspore or embryo sac<sup>1</sup>. Sterility can be expressed in male or female sex track at the phenotypic or genotypic level. Male sterility is, however,

more prevalent than female sterility. Genetically, male sterility can either be sgenic or cytoplasmic or both. At the chromosomal level, many structural aberrations can also lead to sterility. Translocation heterozygosity is one such structural anomaly. Although translocation heterozygotes are as fertile as the normal individuals, many cause sterility. One such example is *Allium roylei* Stearn. *Allium roylei*, belonging to family Alliaceae, is an economically important wild species of North West Himalayas (Figure 1). Besides being used commonly for both culinary as well as medicinal purposes, the taxon is a repository of disease resistant genes<sup>2,3</sup>.

## MATERIALS AND METHODS

The present investigation has been carried out on twenty-five plants of *A. roylei* collected from Bani region (1400 – 1500m) of Kathua district (J&K, India). The plants were established in the experimental beds of Botanical Garden of University of Jammu. These were then tagged, observed and data on plant morphology, fruit and seed set recorded.

### **Pollen viability**

Pollen viability was determined by 2,3,5-triphenyl tetrazolium chloride (TTC) and Fluorescein diacetate (FDA) tests. For this purpose, pollen grains were stained in TTC and FDA on separate slides which were placed in a humidity chamber and incubated in dark for 1-2 hours. Later, these were examined microscopically and scored.

### **Pollen mitosis**

Pollen mitosis was studied from fresh, mature, unopened flower buds by squashing their anthers at appropriate stage in 1% acetocarmine. The slides were made permanent by passing them through n – butyl alcohol and acetic acid series and using Euparal as a mountant.

### **Reproductive output and Seedling establishment**

Fruit and seed set on open pollination were observed in plants growing in the Botanical garden. Number of (a) flowers, (b) fruits and (c) seeds formed per inflorescence were counted and percentages determined for these plants. For this data, seventy-five inflorescences were scanned randomly from twenty-five plants of this species. Seed viability was estimated by allowing seeds to germinate on a moist filter paper inside the petri-dishes kept at room temperature.

## RESULTS AND DISCUSSION

Plants of *A. roylei* collected from Bani, Mendhar and Gourwan regions of Jammu province (J&K) are bulbous herbs bearing linear leaves (Figure 2). Flowering initiates in mid – April with the differentiation of unbranched hollow structure called scape. Each scape terminates into a cymose umbel (Figure 3) and flowering continues till the end of August. An individual plant bears 1-2 inflorescences; each producing 10-108 flowers. Flowers are bisexual, actinomorphic and tristylous. The three flower types i.e. long, medium and short styled ones are borne interspersed with one another in the

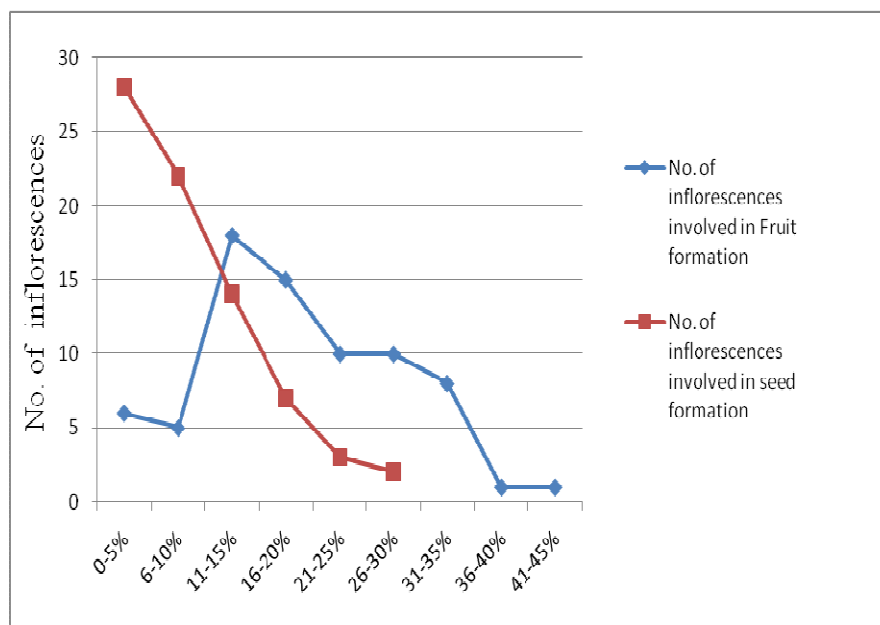
same inflorescence. The long styled ones, however, are uniformly preponderant. Individual flower is white to pinkish – white abscising before as well as after anthesis and majority of these do not transform into healthy fruits (Figure 4, 5). Androecium comprises of six stamens arranged in two whorls of three each. Each anther measuring 2.5x1mm is light yellow, ditheous, basifixed and dehisces laterally. Gynoecium consists of a tricarpellary, syncarpous pistil with a minute, inconspicuous stigma, style and a trilobular ovary. Each locule bears two ovules on axile placentae. Three septal nectaries, one on each septum of the ovary are present. Aggregation of flowers into heads and presence of floral features like tristily, nectaries, enormous pollen count etc are strongly indicative of cross pollination being operative in this plant. A number of insect visitors were found to forage the flowers. Self pollen deposition also seems likely in short and mid styled flowers. This is because similar lengths of stamens (7.8mm) and pistil (7.7mm) and their close proximity in mid styled flowers may lead to self pollination. Similarly in short styled ones, stigma (6.1mm) is placed much below the level of stamens (9.5mm). There is every possibility of the pollen falling from dehiscing anthers onto the receptive stigma.

Although all the cells studied had 16 chromosomes; majority of these exhibit complex associations involving 4 to 16 chromosomes. Only a few had eight perfect bivalents<sup>4</sup>. These anomalies get reflected at anaphase-I. Laggards, bridges coupled with micronuclei formation and unequal segregation characterize anaphase-I. Together these aberrations result in complete meiotic arrest/breakdown. Consequently the microspores formed are largely shriveled/empty/unhealthy. Nearly 0.39 – 26.2% pollen grains are viable as revealed by triphenyl tetrazolium chloride (TTC) test (Figure 6). However, FCR test revealed no viability (Figure 7) implying the effect of multiple associations formed during metaphase-I. Studies on pollen mitosis confirmed the same. Pollen grains

differentiating in a single anther are highly variable in the number and in all likelihood the nature of chromosomes they contain. The haploid complement varied from 1 to 8 (Figure 8 to 11) against the expected 8. Majority of the pollen grains, therefore, are largely hypoploid or highly deficient in genetic content. The female track studies on plants of Bani population revealed  $2n = 16$  in majority of the cells per plant. In addition, few plants with  $2n = 17$  and 19 had also been observed<sup>5</sup>. This means that ovules would be carrying eggs with  $n = 8, 9, 10$  and 11 chromosomes, though there would be a preponderance of those with  $n=8$ . Fertilization of ovules with such pollen grains, if at all, results in progeny with highly variable  $2n$  numbers (9 to 20). Thus, owing to these factors, fruit formation is minimal despite the number of flowers per inflorescence and number of inflorescences per plant being high and this limits seed production as well. The number of flowers per inflorescence ( $n=75$ ) ranges from 10 to 40 and the corresponding fruits formed vary between 0 and 11%, and seeds from 0 to 26.67%. Data regarding the number of inflorescences involved in fruit and seed formation are represented in the figure A. Accordingly; fruit as well as seed abortion rates recorded were 57.69 to 100% and 73.33 - 100% respectively. This leads to extremely reduced fruit – flower and seed – ovule ratios. Whatever little seed is set, most of it is shriveled. Germination registers a further decline and seedlings show a high mortality rate. When such seeds ( $n = 30$ ) are kept for germination, few show radical emergence. Such seedlings survive for 3 to 4 days. Together, contribution to the next generation is almost negligible. Besides this, abortion rates are equally high at the level of flowers. Since plants of various allopatric populations of *A. roylei* gathered from North – West Himalayan region worked out earlier are complex translocation heterozygotes<sup>6,7,4</sup>. This suggests that the genetic material of populations studied so far share a considerable degree of flux. Interchanges are on record in many taxa, however, involvement of entire chromosome

complement in this phenomenon is rare. Some taxa exhibit enormous translocation heterozygosity through multiple interchanges in their entire complement. Despite that a number of factors have contributed to their establishment and successful evolutionary progress/history. Species of *Oenothera* (*Oenothera lamarckiana*) are a classical example. With multiple interchanges, sometimes involving the entire complement, these species set healthy seeds and are as good as normal taxa. This can be attributed to Renner effect<sup>8,9</sup>. Another example is *Chelidonium majus* L. ( $2n = 12$ ) where despite all the 12 chromosomes forming a closed ring during meiosis, the plants exhibit 60 – 70% fertility<sup>10</sup>. It is already known that alternate segregation of quadrivalents and/or multivalents during meiosis results in 50% fertility in the plants. So in order to study the impact of complex chromosomal interchanges on the fruit and seed set in the plants of *A. roylei*, the present investigation has been carried out. Complex translocations coupled with numerous other chromosomal

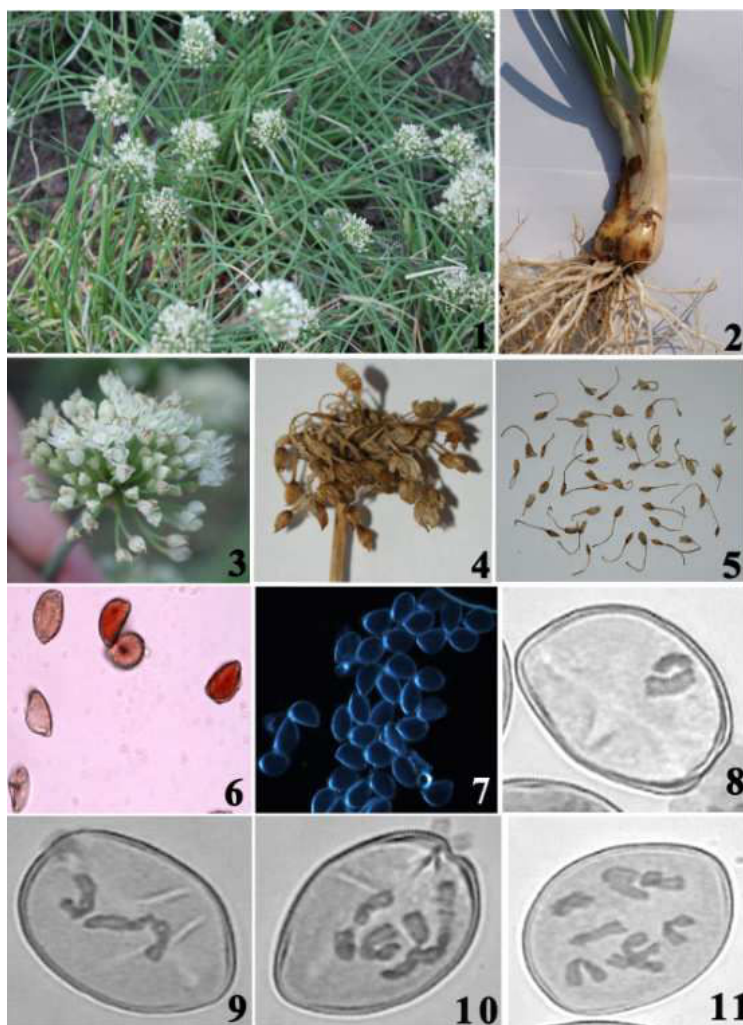
aberrations, in the male track subvert the sexual means of reproduction in the plants otherwise producing large number of flowers. Fortunately the species has an alternative means of reproduction. A single plant of *A. roylei* has the potential to multiply and give rise to 4-5 new underground bulblets. Each such bulblet, when grown separately gives rise to a healthy plant<sup>11</sup>. This strategy ensures clonal multiplication of each genotype albeit at the cost of generating new genetic combinations. Despite the disadvantages accrued to *A. roylei* on account of this reproductive mode, the sterility bottleneck is overcome and replenishment of natural populations is realized. By virtue of this property, extrinsic stress on the plant is considerably lessened. Nonetheless, the species is an enigma as none of the plants collected so far are normal. To what extent are translocations fiddling with the genetic make-up of the species and why, are the questions to which answers are being sought.



**Figure A**  
**Graphical representation of No. of inflorescences involved in fruit and seed formation**

Plate - 1

**Figures 1. Plants of *A. roylei*, 2. Underground bulbs, 3. an inflorescence, 4 & 5. aborted fruits 6 & 7. Pollen grains stained with TTC and FDA respectively 8, 9, 10 & 11. Pollen grains with one, two, four and eight chromosomes respectively.**



## REFERENCES

1. Davis, B. M. 1923. Pollen- and Seed-Sterility in hybrids. *American Journal of Botany*. 10(9), 462-467.
2. de Vries, J. N., Wietsma, W. A. and Jongerius, M. C. 1992. Introgression of characters from *Allium roylei* Stearn into *A. cepa* L., In : P. Hanelt et al., (ed.). *The Genus Allium – Taxonomic problems and genetic resources IGCP, Gatersleben, Germany*. pp. 321-325.
3. Scholten, O. E., van Heusden, A. W., Khrustaleva, L. I., Burger-Meijer, K., Mank, R. A., Antonise, R. G. C., Harrewijn, J. L., Van haecke, W., Oost, E. H., Peters, R. J. and Kik, C. 2007. The long and winding road leading to the successful introgression of downy mildew resistance into onion. *Euphytica*. 156(3), 345-353.
4. Kohli, B. and Gohil, R. N. 2011. Is *Allium roylei* Stearn still evolving through multiple interchanges? *The Nucleus*. 54(1), 19 – 23.

5. Sharma, G. and Gohil, R. N. 2011. Occurrence of differential meiotic associations and additional chromosomes in the embryo-sac mother cells of *Allium roylei* Stearn. *Journal of Genetics*. 90(1): 45-49.
6. Sharma, G. and Gohil, R. N. 2003. Cytology of *Allium roylei* Stearn 1. Meiosis in a population with complex interchanges. *Cytologia*. 68(2), 115-119.
7. Kohli, B. and Gohil, R. N. 2009a. Chromosomal heteromorphism in a population of *Allium roylei* Stearn. *The Nucleus*. 52(1, 2), 1-8.
8. Cleland, R. E. 1972. *Oenothera* : Cytogenetics and Evolution. Academic press, New York.
9. Swanson, C. P., Merz, T. and Young, W. J. 1990. *Cytogenetics: The Chromosome in division, inheritance and evolution*, 2<sup>nd</sup> Ed. Prentice – hall, New Delhi.
10. Pilquin, I. P. 1981. An unusual case of a Complex heterozygote presenting no taxonomical problem in *Chelidonium majus* L. (Papaveraceae). *Experientia*. 37 : 341-342.
11. Kohli, B. and Gohil, R. N. 2009b. Need to conserve *Allium roylei* Stearn: a potential gene reservoir. *Genet Resour. Crop Evol.* 56: 891-893.