

SEED BIOLOGY OF *ARTEMISIA MARITIMA* L. AN OVEREXPLOITED MEDICINALLY IMPORTANT SPECIES IN NORTH WEST HIMALAYAS

Jyoti Parihar, Uma Bharti, Irshad Ahmed Hamal* and Namrata Sharma**

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

*Baba Ghulab Shah Badshah University, Rajouri, J & K

**e-mail—phyllanthus@rediffmail.com

Abstract: *Artemisia maritima* of family Compositae is an endangered perennial shrub with localized distribution because of its being highly habitat specific (Parihar *et al.*, 2011). Plants forming natural populations in Kishtwar Himalayas in J&K state, India, show high fruit and seed set in open fields ($x = 83.7\%$). Details of floral structure and events of floral biology reveal the species to be outcrossed, although it has the capacity to set seeds by selfing also. The same is accomplished through geitonogamy and by self pollen germinating at the point of nectary capping the ovary (Parihar *et al.* 2009). Seeds of the species, one per fruit, is with straight embryo and unique in being of two different colors, grey and brown. Both types are alike morphologically but differ in weight. On a moist filter paper, the %age of seeds germinating averages 34.33% for grey seeds and 47.5% for brown seeds. Most of the seedlings emerging out of these seeds however fail to establish. These observations reveal the manifestation of inbreeding depression in the species. This outcrossed species is supposedly forced to set seed by selfing due to squeezing of populations due to overexploitation and by a single individual occupying considerable area due to perennation for several years.

Keywords: *Artemisia maritima*, Seed

INTRODUCTION

Since last two decades, studies on reasons leading to squeezing of populations of some plant species have started bringing into focus several intrinsic problems too. The main among these are sluggish reproduction, inability to compete with other species of the community, prolonged seed-seed cycle, seedling mortality etc. Species that are rare and have small population size, have been shown to suffer from inbreeding depression, also leading to decline in fitness. All these intrinsic factors have been cited to contribute heavily in making a species vulnerable to extinction (Frankel and Soule, 1981; Levin, 1983; Ledig, 1986; Lacy, 1992; Bruna and Kress, 2002).

Artemisia maritima of Family Compositae has gained importance as the source of a drug called Santonin effective in its action on roundworms (Kaul, 1984). The species is used as an important fodder plant and has several other medicinal and aromatic uses also. All these above cited uses have helped on one hand in its enlistment as an important minor forest product in J&K state, but on the other hand have led to its overexploitation leading to considerable squeezing of its populations. This overexploitation has prompted some workers to place this species in endangered category (Kaul, 1996). The species is temperate with localized distribution. In J&K state, India it is restricted to Kishtwar Himalayas.

The plant perennates in winter via horizontal creeping root stock sprouting in the month of March. It remains in the vegetative state for 5-7 months and subsequently flowers in October/November when the day temperature fluctuates between 25°C and 30°C. Flowering is profuse and a single plant on an average produces 28,709±1964 capitula, each consisting of 6-13 homomorphic florets (Figure 1a).

Seed set is initiated during December and averages 83.4% on open pollination. Detailed studies on floral phenology and pollination strategies in this species suggest it to be predominantly cross pollinated but with the ability to produce seeds by geitonogamy also. Pollen germination at the point of nectary is also an interesting mechanism in this species and is responsible for some amount of seed set (Parihar *et al.* 2009). We observed fruit and seed set %age on open pollination and after unassisted selfing and also collected seeds from plants growing in district Kishtwar of J&K state during 2005-2009. The present communication deals with interesting observations made on some aspects of seed biology of this species.

MATERIAL AND METHOD

Populations of *Artemisia maritima* L. were identified and tagged in four different localities in Kishtwar town of J&K state. Individual plants were tagged at each place for observations on plant morphology, flowering phenology and fruit and seed set.

Fruit and seed set

Fruit and seed set on open pollination were observed in plants growing open in the field. Number of flowers/capitulum, number of fruits set/capitulum and seed set/capitulum were counted for these plants.

%age fruit and seed set on open pollination was calculated as under:

$$\% \text{age fruit / seed set / inflorescence} = \frac{\text{Total fruit/seed count/inflorescence}}{\text{Total number of flowers/inflorescence}} \times 100$$

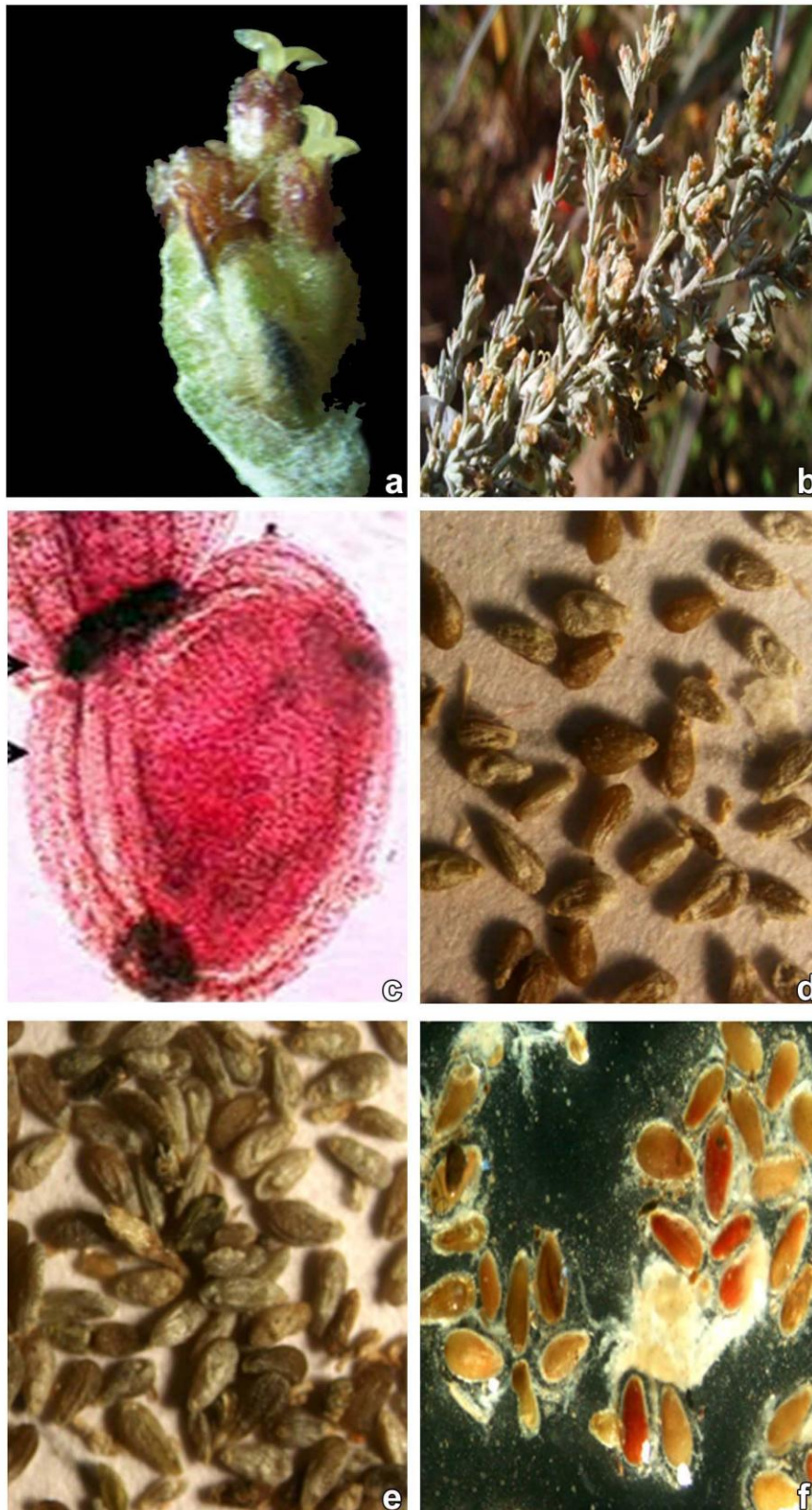


Figure 1a-f - a. A Capitulum of *Artemisia maritima* bearing florets with bifid extruding stigmas. b. Axillary spikes of *Artemisia maritima* bearing floral heads. c. An epigynous nectary capping one ovuled ovary at the base of style. d-e. Brown and grey seeds of *Artemisia maritima* f. Seed viability as revealed by TTC(2,3,5-triphenyl tetrazolium chloride) test.

Bagging experiment

Single, 3-6 inflorescences together and whole inflorescence branches were bagged on different plants with butter paper bags of appropriate size. These were later checked for fruit and seed set by geitonogamous pollination using the formula as elaborated for open pollination. Stigmas were decapitated in some florets as soon as they came out. These florets were then bagged. Data on fruit and seed set were then recorded for these inflorescences and also for individual flowers in these inflorescences to check fruit set by pollen germinating at the point of nectary.

Seed viability and seedling establishment

The viability of seeds was estimated by allowing seeds to germinate on a moist filter paper inside the petridishes kept at room temperature and in garden soil in experimental plots. Viability of seeds was also tested by 2,3,5-triphenyl tetrazolium chloride (TTC) test. For the same, seeds were placed in a petridish in TTC solution. The petridish was later placed in a humidity chamber and seeds were incubated in dark for 2-3 hours.

Seed weight and size

Seeds were kept in vials (n= 150) for determining weight and size. Seed size was determined using microscope equipped with ocular micrometer and seed weight was determined on electrical balance in laboratory.

RESULT AND DISCUSSION

Plants of *A. maritima* are perennial, perennating by horizontal rhizomes that sprout in the month of March to produce aerial offshoots. Each aerial offshoot attains a height of 40-71 cm. Flowering occurs after 5-7 months of vegetative phase. The flowers arise in axillary spikes terminating into heads (Figure 1a). Single plant produces on an average $28,709 \pm 1964$ capitula (Figure 1b) each of which is homomorphic and contains 6-13 bisexual florets ($x = 8 \pm 2.0$). Each floret contains five syngeneious stamens and a single pistil with bicarpellary syncarpous inferior ovary which is unilocular and one ovuled (Fig. 1c). Florets are protandrous, with anther dehiscence commencing 4-5 days before the onset of stigma receptivity.

Fruiting in *Artemisia maritima* initiates during early November and continues till early December. Fruit is cypsela; a dry indehiscent, one seeded fruit developed from a bicarpellary, syncarpous, inferior, unilocular ovary. Each fruit remains enclosed by persistent bracts. Fruit and seed set in open field is quite high and varies between 50-100%, it averages 83.4%. High investment in flower production and

high seed set on open pollination portrays that *A. maritima* is reproductively efficient and has no apparent bottlenecks in its sexual reproduction. Data on unassisted pollination however reveals an interesting pattern.

Since individual flowers are too small to be checked for seed set on different pollination treatments, data on seed set was collected per inflorescence and different treatments were tried. Bagging of single inflorescence per branch on 25 different individuals resulted in low seed set varying between 11.1 to 22.2% ($x = 16.50\%$). When three inflorescences were bagged together on 25 different individuals, seed set improved a bit but was still very low. It ranged from 16.6- 33.3% and averaged 25.71%. Random bagging of the six inflorescences of a branch together was done in 25 different individuals. Seed set on these inflorescences was still low and varied between 25-33% ($x = 30.62\%$). In 25 different individuals, single inflorescence was selected/ individual at random and in all the flowers of these inflorescences ($x = 6.440 \pm 0.209$), stigmas were decapitated as soon as they came out of the floret i.e. before these diverged their lobes. The seed set on these florets averaged $x = 21.09 \pm 21.8$. Except few seeds which were shrivelled and curved, all seeds resembled the ones set on open pollinated flowers.

Foregoing account reveals a reduction in seed set on unassisted selfing; the reduction being inversely proportional to the number of inflorescences bagged together. This suggests both inbreeding depression as well as availability of sufficient pollen as factors for this reduction. The data also clearly reveals that although *A. maritima* is cross-pollinated, it keeps a provision for self-pollination provided for some reasons, cross pollen transfer is not possible.

Seed one per fruit is brown or grey in colour (Figs. 1d and e). It bears a straight embryo and is small in size averaging 1.101×0.51 (mm). Seeds of both the colours i.e. grey and brown are similar in overall structure but differ in weight. The dry weight of brown seed averages 0.002 gm, while the dry weight of the grey seed is almost half (0.001gm). Seed viability was tested by 2,3,5-triphenyl tetrazolium trichloride (TTC) test. Ten samples of 50 seeds each were tested and an average of 28.20 ± 1.17 turned pink on treating with TTC. The viability as tested by TTC test thus averaged 56.4% (Fig. 1f).

Seed germination in *Artemisia maritima* takes 6-8 days under lab conditions. Seeds do not show any dormancy as they germinate without requiring any resting period. In experimental plots containing a mixture of sand and garden soil at Govt. Degree College, Kishtwar, seed germination averaged 60%. Out of 10 samples of 50 seeds sown, 30.8 ± 1.9 showed the emergence of seedlings. More than 50% of these seedlings however failed to establish.

In another set of observations, grey and brown seeds were segregated into 6 heaps of 200 seeds each (3 grey and 3 brown) and their germination was

observed on a moist filter paper in the lab. Grey and Brown seeds showed significant differences in their germination %age. Germination of grey seeds averages 34.33% while that of brown seeds was more and averaged 47.5%. These thus showed apparent differences in their viability. Although displaying difference in color and viability, seeds in *Artemisia maritima* cannot be distinctively termed as of two different types as both resemble in size and overall morphology. This is unlike many other composites like *Rimerophotheca pluvialis*, *Felicia heterophyll*, *Chrysanthemum segetum*, *Calendula officinalis*, *Crepis dioscoridia* and *Syndrell noliflora* (Babcock,1947; Zohary, 1950; Levyna, 1972) where achenes display differences in being flat and winged as an adaptation for dispersal by wind.

All the above observations indicate that *A. maritima* has bottleneck in its life cycle at the point of seed germination. Although abundant investment is made in the production of enormous amount of seeds per plant, about half of this lot is viable and shows germination. Whether this hurdle is manifested again at the time of seedling establishment needs to be detailed out in lab condition, although inclination towards it is seen in field. Low seed germination as well as low seedling survival seems to cut down the high rewards of sexual reproduction in this species.

REFERENCES

- Babcock, E. B.** (1947). "The Genus *Crepis*." California University Press, Berkely and Los Angeles.
- Bruna, M. and Kress, W.J.** (2002). Habitat fragmentation and the demographic structure of an Amazonian understory herb, *Heliconia acuminata*, *Conservation Biology* **16** : 1256-1266.
- Frankel, O. H. and Soule, M.E.** (1981). *Conservation and Evolution* – Cambridge University Press.
- Kaul, M. K.** (1996). Strategies for sustainable use of medicinal plant resources in Western Himalaya In S K Jain (ed) *Ethnobiology in human welfare*, Deep publication, New Delhi. pp. 284-286.
- Kaul, M. K. and Bakshi, S. K.** (1984). Studies on the genus *Artemisia* L in North West Himalaya with particular reference to Kashmir. *FolioGeobotanica et Phytotaxonomica* **19**.
- Lacy, R. C.** (1992). The effects of inbreeding on isolated populations are, minimal viable populations predictable? In P L Fiedler & S K Jain (eds), *Conservation Biology*. Chapman and Hall, New York NY. pp. 277-276.
- Ledig, E.T.** (1986). Heterozygosity heterosis and fitness in outbreeding plants . In M.E. Soule (ed.), *Conservation Biology: The Science of Scarcity And Diversity*. Sinauer Associates, Sunderland M.A. pp. 446-461.
- Levin, D.A.** (1983). Immigration in plants: an exercise in the subjunctive. In C.M. Schonewald – Cox S. M. Chambers, B. Macbryde & W. L. Thomas (eds.), *Genetics and Conservations*. Benjamin – Cummings, Menlo Park CA. pp. 242-260.
- Levyna, M R.** (1972). The Rehnosterbush , *Veld and Flora* (2): 7-9.
- Parihar J.; Hamal, I. A. & Sharma, N.** (2009). Dual pollen germination in *Artemisia maritima* L. *Current Science*. **97**: 24-25.
- Parihar J.; Hamal I. A. & Sharma, N.** (2011). Restricted distribution of *Artemisia maritima* L., an endangered plant in Kishtwar Himalayas J & K – Role of edaphic factors. *National Academy Science Letters* **34** 317 – 320.
- Zohary, M.** (1950). Evolutionary trends in the fruiting head of Compositae. *Evolution* **4**:103-109.