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Improvement of Sexual Destination in *Atropa acuminata* Royle (Solanaceae)-A Critically Endangered Medicinal Plant of Northwestern Himalaya

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Abstract: Good seed set is no guarantee of absolute sexual destination in plants. Seed viability and seed vigour are crucial phases in the life cycle of every sexually reproducing plant. The present study was an attempt to improve the sexual destination-the germination and seedling survival of *Atropa acuminata* Royle (Solanaceae), an endemic and extremely restricted sub-alpine medicinal plant of North West Himalayas under *ex situ* conditions at (1580 m) with an aim to develop a successful germination protocol and agrotechnique in order to revegetate disturbed areas. Among various treatments given to the seeds, GA_3, Scarification, warm water treatment and chilling at 4°C for 90 days were found to be most effective with percentage germination of 73.3±18.80, 79.95±9.40, 66.6±6.6, 45±7.07 (X±SE), respectively. The results reveal that the seeds do not germinate unless specific environmental signals or events occur which trigger the genetic and hormonal response of the seeds thereby facilitating their germination. The diversity and the extent of the dormancy mechanisms encountered here suggest that under harsh conditions, natural selection may favour seeds with a genetic system for dormancy and delayed germination. A relation was observed between seed size/weight,%age germination and subsequent seedling survival. Seedling survival is also effected by specific habitat requirement and stiff intra and inter-specific competition particularly the whimsical behaviour of *Sambucus wigthiana* (an alien species which grows in the vicinity of *Atropa*) is beyond the ken of *Atropa*, adding fuel to the already burning candle apart from habitat fragmentation and herbivory.

Key words: *Atropa acuminata*, sub-alpine herb, environmental signals, hormonal response, germination, interspecific competition, survival

INTRODUCTION

The genus *Atropa* consists of four species distributed in the Mediterranean region, South Europe and Asia (Anonymous, 1948). Of these *Atropa belladonna* has long been used as a reputed drug in Europe and is still regarded as one of the few indispensable drugs of the plant origin (Anonymous, 1948). Because of the mydriatic action of the leaf juice when introduced on eyes, Italian and Spanish ladies use it frequently as a means of imparting a seductive appearance to their eyes.

However the species of the genus found in Asian continents is *A. acuminata*, which is regarded to be more or less equivalent to *A. belladonna* in terms of chemical constituents (Anonymous, 1948). In North West Himalaya it is distributed in Kashmir, Muzzafarabad and Chakrata (Anonymous, 1948; Dhar and Kachroo, 1983). The drug Atropine and Hyoscyamine extracted from the plant acts as stimulant to the sympathetic nervous system and are employed as antidote to Opium (Anonymous, 1948; Kaul, 1997). Internally it is used for the treatment of whooping cough and asthma, externally as liniment to relieve neurological pain (Kaul, 1997). Atropine has a stimulatory effect on the circulatory and respiratory system (Kaul, 1997). Ranking at the top of medicinal plant inventory from North West Himalaya, it figures among 59 critically endangered taxa and negative list of exports in India and has been prioritized for immediate conservation and large multiplication (Choudary and Rao, 1998a,b). Owing to the immense medicinal value, the plant is being indiscriminately exploited on a large scale. Unabated as the plant extraction continues to be, far are not days when this precious legacy will be loss forever. It is indeed a crisis situation for the species which calls for the salvage of whatever is left. If not rescued now, irretrievable loss of this precious legacy from the globe will be the eventual and inevitable consequence.

To develop a suitable prescription and panacea for this malady, understanding of reproductive biology, breeding behaviour and seed biology is regarded to be of nuclear importance as well as the central element (Wafai and Nawchoo, 2001). This will not only help in planning strategies for the effective and efficient conservation,

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validity and management but also pave the way for sustained generation and regeneration of raw material. This inturn can be reintroduced into natural habitats for reallocation of bioresources and for usage by pharmaceutical industries in the days to come.

Seed dispersal and germination are the phases of reproductive cycle that are typically of great significance for the species fitness. Variations in seed dispersal efficacy or seed viability are often interpreted as a reflecting adaptation to specific ecological conditions (Venable and Lawlor, 1980; Grine *et al.*, 1981; Martin *et al.*, 1995; Nishitani and Masuzawa, 1996). Clearly the micro site occupied by the seed may strongly influence its probability of germination and subsequent survival. Indeed species that lie in highly specific habitats often produce seeds with highly specialized adaptations (Venable and Lawlor, 1980; Grime, 1979; Pickart, 1988). Further more, many studies have demonstrated that seed size and/or weight may be a good predictor of various performance variables including germination capacity (Schaal, 1960; Dolan, 1984; Hendrix, 1984; Stanton, 1984; Wulff, 1986a; Marshell, 1987; Naylor, 1993) resistance to intra and inter-specific competition (Wulff, 1986b; Mazer, 1989; Houssard and Escarre, 1991) dormancy period (Stamp, 1990), distance dispersed with respect to mother plant (Augspurger and Franson, 1986) and seedling survival (Schaal, 1980; Howe and Richter, 1982; Stanton, 1984; Weller, 1985; Wulff, 1986a; Marshell, 1987). Any deviation from the normal mode of sexual reproduction together with the continued adverse conditions, herbivory, habitat degradation, extraction and stiff inter and intra specific competition threatens the survival and existence of the species. Of these questions of theoretical interest and as pointed by Schemske *et al.* (1994) and Bernardello *et al.* (2001) detailed information on the different aspects of reproductive cycle of endemics, rare and threatened medicinal species may contribute to improve understanding of the phenomenon of rarity and at the same time assist conservation management decisions for the species.

Atropa acuminata, is one such important medicinal plant of North West Himalaya facing the above mentioned conditions wherein the seedlings of a population neither resist the unfavourable climatic conditions nor tolerate the herbivore. They also compete with each other while in the battle for resource utilization. All these factors have actually brought the species near the brink of extinction and is categorized as critically endangered (CR) (Mamgain *et al.*, 1998). The species decline, in addition to economic fall out also has a bearing on the other functional aspects of the ecosystem in particular and environment in general (Wafai and Nawchoo, 2001). The present study was carried to rescue the crisis situation of the species with the objective of developing a suitable protocol for seed based commercial cultivation of the species at low and easily approachable altitudes. This will help in conservation and multiplication of the endangered taxon thereby facilitating its mass multiplication which inturn will pave the way for sustained generation and regeneration of raw material for reintroduction into natural habitats and for commercial usage by drug industries in the future.

MATERIALS AND METHODS

Few natural populations were selected and the various performance variables including the competition both intra-specific and inter-specific for resources, space and light was constantly observed for three consecutive years. To unravel the modes of propagation operative in the species several plants apart from populations were earmarked at the end of the first growing season. In the subsequent growing season they were carefully dug out and analysed keenly for the structure and number of perennating shoots.

Occurrence of the seedling in the natural population served as an indicator of successful development of seedlings from seeds (sexual propagation). Mature seeds of *Atropa acuminata* of the same age group (cohort) but of different sizes were collected (September-October) from the specific habitats of the species viz. Gulmarg range of Kashmir Himalaya (2250-2450 m; 34°04N/74°20 E). The seeds were stored in paper bags away from light at 8°C till March. The seeds were subjected to tetrazolium topography test (Basu and Sur, 1988) to check the viability of seeds. Before setting for germination the seeds were treated with 0.5% Mercuric chloride for 5 min and then with 70% ethanol for 1 min and afterwards washed thoroughly with distilled water. The hormonal and chemical treatments given to the seeds include GA_3, Kinetin, Thio-Urea, KNO_3 (0.125 nM, 0.5, 0.75 and 1.0 mM); Concentrated H_2SO_4 and HNO_3 and the physical treatments including warm water treatment and chilling of 30, 60, 90, 105 and of 120 days at 4°C.

For each treatment four replicates were used, two kept in light and two in dark, each with a set of control to compare germination efficiency. Percentage germination was calculated as:

$$(\%)\text{ age germination} = \frac{\text{No. of seeds germinated}}{\text{Total No. of seeds kept for germination}} \times 100$$

RESULTS AND DISCUSSION

The geographic range of *A. acuminata* now occupies less than 30 km^2 in Kashmir Himalayas from

Tangmarg (1900 m asl) to Gulmarg (2500 m asl) and to few kilometers in Daksum range (2000-2450 m asl) excluding Muzafarabad, comprising only a few scattered populations. The observation is based on the three years extensive survey and exploration of Kashmir Himalaya. The habitat falls in temperate climatic zone within the geographical coordinates of 34°04′ N/74°20′ E (latitude/longitude). The average rainfall ranges from 90-150 cm with a summer drought of 1-2 months. The mean annual temperature ranges between 7.5-11.2°C but during the winter months temperature may drop down to -10°C. It is an area with a mosaic types of habitats including, cultivated land, small tribal villages, home of various plants including *Verbasicum thapsus*, *Cirsium wallichii*, *C. falconuriai*, *Sambucus withiana*, *Poa annua*, *Urtica dioica Ceneturea* sp., *Parthenium* sp. apart from pine trees. This observation is the out come of extensive exploration of the whole Kashmir Himalaya including Ladakh from June 2002 to November 2005. *Atropa acuminata* Royle, propagates both by vegetative and sexual means. The vegetative propagation is through the development of underground rhizome, while the sexual reproduction procedes through seeds. As and when the seeds disperse from the berries they germinate immediately on a suitable habitat from the last week of September to Second week of October and those which remain inside the wall of berry starts to germinate in the last week of March to mid April. Due to the unfavourable conditions at the alpine and sub-alpine regions. The young seedlings produced in September or October usually suffer winter frost, while the seedlings produced in spring become the victims of predation and competition. Due to fragile nature of the habitat together with unique and high value medicinal properties, the species is facing the onslaught of indiscriminate and ruthless exploitation, with the result its populations in entire North West Himalayan range are witnessing speedy decline and dwindle both in size and number.

The flowers of *Atropa acuminata* is being pollinated by *Apis indica* belonging to order Hymnoptera. The plant during the course of evolution has learnt and evolved a mechanism to avoid the inbreeding through temporal separation of two sexes. The species is protogynous. The stigmas almost loose their receptivity by the time pollen is shed. Viability of pollen ranges from 95 to 98%, culminating exceptionally into a good seed set (93.6-138.9 berries per plant and 10525.22-15619.3 seeds per plant, each value given here is the mean of 15 plants). The seeds of *Atropa acuminata* show a viability of 95-97% with tetrazolium chloride. The present study conducted on the seed germination at 20-22°C, revealed that the seeds do not germinate in dark, pointing towards the photoblastic nature of the seeds. Freshly collected seeds though germinate immediately, but the seedlings when transplanted at Herbal garden Kashmir University (1580 m) collapsed due to the sub-zero temperatures from November onwards. Similar observations were made by Brink (1964), on arctic and alpine plants thereby suggesting and supporting the view that alpine and sub-alpine seed dormancy may have evolved in response to a temporally unpredictable environment. Seed germination in such cases can be limited to brief periods in the summer when soil water is available (Bliss, 1971). GA_3 (0.5 mM) resulted in 73.3% of germination as against the control 33.3% germination. Almost similar results were obtained by Prassad (1999) and Ganie and Nawchoo, (2002), on *Podophyllum hexandrum* and *Arnebia benthamii* respectively. From the observations it becomes clear that the germination of the seeds is either impaired due to impermeability of seed coat or else due to the seed coat inhibitors as argued and experimentally demonstrated by Amen (1966) and by Ganie and Nawchoo (2002) on *Arnebia benthamii*,-an alpine herb. Further seed size and seed weight has also been found to be an important factor for germinability, subsequent seedling survival and other various performance variables viz. dormancy period, inter- and intra-specific competition (Shaal, 1960; Dolan, 1984; Hendrix, 1984; Marshall, 1987; Naylor, 1993; Mazer, 1989; Houssard and Escarre, 1991).

On the basis of seed size and weight two types of seed samples were collected from a selected habitat (i.e., Gulmarg). From each sample 50 randomly selected seeds were weighed, one of the sample weighed 0.06 g 50 seeds and other 0.04 g. A type of positive correlation was observed between the seed size to germination and subsequent seedling survival. Besides Scarification (79.95±9.40%), warm water treatment (66.6±6.6), GA_3 0.5 mM (73.3±18.80), chilling (at 4-5°C for 90 days) resulted in 70% germination and two nitrogenous compounds viz. thio-Urea with highest germination in 1 mM (66.6±10.4) and KNO_3 with highest germination (46.65±9.40) at 1 mM concentration. The acid treatment was found to be detrimental for the seed germination of this species. The seedlings thus raised when transplanted in the Herbal garden Kashmir University and spaced in rows and 9 inch apart showed 82-88% survival with the seed sample weighing 0.06 g 50 seeds while those of 0.04 g 50 seeds showed 56-59% survival.

The various performance variables of *A. acuminata* were observed by tagging one of the natural populations which were spreaded over an area of 7.2 m: 5.2 m (length: breadth), comprising 26 individuals of *Atropa acuminata* and 37 individuals of *Sambucus wightiana*. The competition between the two was observed in terms of various quantitative and qualitative features including solar energy. Another isolated population of *Atropa acuminata* growing without *sambucus*, served as control. These experiments were also carried out under *ex situ* conditions at Herbal garden of Kashmir University (1580 m). The results reveal that the individuals of *Atropa* do suffer because of interspecific competition with *Sambucus* and face stiff challenges for its existence and survival. The individuals of *Sambucus* do reduce the various performance variables including germination capacity and survival of seedlings of *Atropa acuminata* to almost its half (Fig. 1 and 2). This in turn was supported and confirmed by the transplanted experiments were the plants when grown alone on loamy soils were found to be more vigorous with high seedling survival than when grown in close proximity with *Sambucus wightiana.* Apart from competition, herbivory of seedling, habitat fragmentation adds the fuel to the already burning candle. Under natural conditions only a few seedlings were seen in spite of efficient seed set relative to other plants of the same habitat excluding *Sambucus wigthiana*. The seedling survival in nature ranges from 15-22%. The seed set is so good that even if only 20-25% of the seeds get germinated, one should see thousands of seedlings in nature annually. In nature this never happens, probably because of the poor dispersal mode of the species together with stiff competition with an exotic weed *Sambucus wigthiana* (Fig. 3).

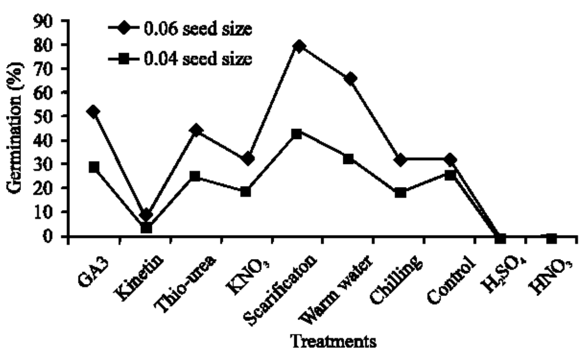

Fig. 1: Seed size and mean (%) germination

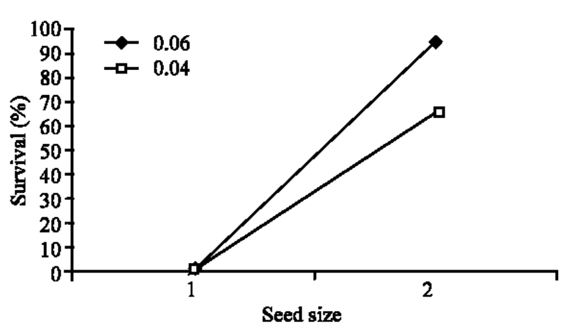

Fig. 2: Comparative seedling survival

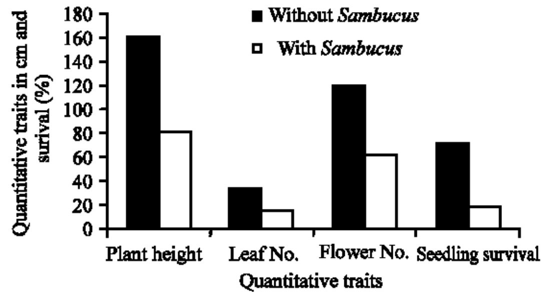

Fig. 3: Competitive ability of *Atropa*

From the observations one can easily pin point the bottle necks of the species decline and also that protection of natural habitats (*in situ* conservation) is the need of hour and time to join hands with the global slogan-to preserve and conserve the bio-resources especially economically important plants in general and threatened gems in particular. Further it becomes clear that the proper management and the maintenance of the bioresources will help in developing conservational strategies. The basic requirement is to collect the vegetative as well as sexual propagules at proper stage, store them properly and start propagation protocol at specific time and under specific conditions.

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