in spontaneous mutants. The changes could have concerned point mutations in genes as well as greater chromosomal aberration[1,15].

Our study clearly indicated that RAPD markers could be effectively used for genetic diversity studies among radiomutants of Indian origin at genomic level. The results obtained suggested that by using RAPD molecular markers the newly evolved chrysanthemum cultivars can be easily differentiated from their parents. This would be a useful tool in identifying and protecting them from possible infringements in future.

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Seed germination behaviour of some medicinal plants of Lahaul and Spiti cold desert (Himachal Pradesh): implications for conservation and cultivation

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Seed germination/dormancy status of seven plant species of reasonably high medicinal value from the cold desert region of Lahaul and Spiti (Himachal Pradesh, India), namely *Podophyllum hexandrum*, *Hyoscyamus niger*, *Inula racemosa*, *Bunium persicum*, *Carum carvi*, *Saussurea costus* and *Rheum australe* was determined. These species are under pressure due to overexploitation from their natural habitats. Seeds of *P. hexandrum*, *H. niger*, *I. racemosa* and *B. persicum* were completely dormant at harvest. The efficacy of chilling, acid scarification, KNO_3 and GA_3 treatments for germination improvement was tested. The most effective treatments in different species were – *P. hexandrum*: $H_2SO_4/10^{-3}$ M GA_3; *H. niger*: 10^{-3} M GA_3; *I. racemosa*: chilling; *B. persicum*: chilling; *C. carvi*: chilling; *S. costus*: chilling; *R. australe*: 10^{-3} M GA_3. The presence of chemical inhibitors in dormant seeds, assessed as the degree of inhibition of seed germination of *Triticum aestivum* and *Brassica juncea* was indicated in *B. persicum* and *C. carvi*. The seedlings derived from seeds exposed to the various treatments performed well when grown in a glasshouse. The data have implications for conservation and cultivation of the species studied.

Keywords: Cold desert, conservation, cultivation, medicinal plants, seed germination.

THE demand for medicinal plants has increased globally due to the resurgence of interest in and acceptance of herbal medicine. Most of the demand is being met through collection of large quantities of medicinal plants and plant parts from wild populations. The methods of extraction employed are almost invariably crude and unsystematic. As a consequence, the rates of exploitation may exceed those of local natural regeneration. Also, the natural habitats are fast depleting[1,2]. The Indian Himalayan Region (IHR) is a rich reservoir of biological diversity in the world. Lahaul and Spiti (lat. 31°44′57″–32°59′57″N and long. 76°46′29″–78°41′34″E) constitute parts of cold desert in Himachal Pradesh (India) within the IHR[3]. This region, home to many a high-value medicinal herbs, has a rich local tribal tradition of herbal medicine. Interestingly, the high altitude populations of medicinal plants such as

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Table 1. Brief description of plant species studied

Name/family	Local name	Habit	Altitude of the collection site (m asl)	Use
Podophyllum hexandrum Royle (Berberidaceae)	Omoshey	Perennial herb	3850	Rhizome, roots, fruits used as anticancer agents and in the treatment of ulcers, hepatic disorders, wounds/cuts, tuberculosis and constipation
Hyoscyamus niger Linn. (Solanaceae)	Dhanduru	Biennial herb	3050	Dried leaves, flower tops and seeds used as astringent, aphrodisiac and sedative agents and also for treatment of muscular pains, toothache and asthma
Inula racemosa Hook. F. (Asteraceae)	Manurucha, manu	Perennial herb	3000	Roots used as anthelmintic, antiseptic, anti-inflammatory and diuretic agents
Bunium persicum (Boiss.) B. Fedtsch. (Apiaceae)	Kala jirah	Perennial herb	3100	Fruits/seeds used as spice, carminative, back pain, liver problem and stimulant
Carum carvi Linn. (Apiaceae)	Gonyor, jirah	Biennial or annual herb	3000	Fruits/seeds used as spice, carminative, back pain, liver problem and stimulant
Saussurea costus (Falc.) Lipsch. (Asteraceae)	Kuth	Perennial herb	3000	Roots used as anti-arthritic, antiseptic, aphrodisiac, carminative and digestive agent
Rheum australe D. Don. (Polygonaceae)	Archo	Perennial herb	3350	Roots, stem and petioles used as appetizer, astringent and in the treatment of asthma, bronchitis, eye diseases, piles, etc.

Table 2. Effect of various treatments on seed germination behaviour and seedling survival percentage of some medicinal plants

Plant species	Constraints in germination	Effective treatments	Ineffective treatments	Inhibitory treatments	Per cent survival
P. hexandrum	Complete dormancy	H_2SO_4/GA_3 comb. > GA_3	KNO_3, chilling, acid scarification	–	84
H. niger	Complete dormancy	GA_3 > chilling > KNO_3	Acid scarification	–	6**
I. racemosa	Complete dormancy	Chilling > GA_3	Acid scarification, KNO_3	–	80
B. persicum	Complete dormancy	Chilling only	Acid scarification, GA_3, KNO_3	–	0**
C. carvi	Low germination	Chilling > GA_3	Acid scarification	KNO_3	72
S. costus	Moderate germination (max. 68%)	Chilling > GA_3 > KNO_3	–	–	83
**R. australe*	High germination (max. 89%)	Marginally improved with GA_3, KNO_3	–	–	88

*8 month stored seeds used; **Did not grow beyond 3–4 weeks.

those from Lahaul and Spiti are known to yield markedly superior active principles compared to their lower altitude counterparts. For example, Sharma *et al.*[4] demonstrated much higher podophyllotoxin content in *Podophyllum hexandrum* collected from Jalori Pass (8000 ft) than in the Palampur (4000 ft) counterpart. Several high-value medicinal plant species of the Lahaul and Spiti region are threatened, their status ranging from low-risk-near-threatened to critically endangered[5–8].

There is thus an urgent need to develop and implement regeneration/conservation strategies for exploited medicinal plant species. A simultaneous development of easy-to-employ means of *ex situ* propagation of the species concerned would encourage their cultivation, thereby considerably easing the pressure on natural habitats. The common means of regeneration and propagation of medicinal plants include seed-based, clonal and micropropagation methods. Seed-based multiplication is the most effective, realistic and convenient means for most species. The objective of the present study was to determine the seed germination behaviour of seven plant species of known medicinal value from the cold desert area of Lahaul and Spiti. A brief taxonomic description and medicinal uses of these are given in Table 1. Further details are available elsewhere[9,10].

Seeds of *Podophyllum hexandrum*, *Hyoscyamus niger*, *Inula racemosa*, *Bunium persicum*, *Carum carvi* and *Saussurea costus* were collected from Patan valley (3000–3850 m asl) and those of *Rheum australe* were collected from the Spiti valley (approx 3350 m asl) during July to September 2002–03. Both these locations belong

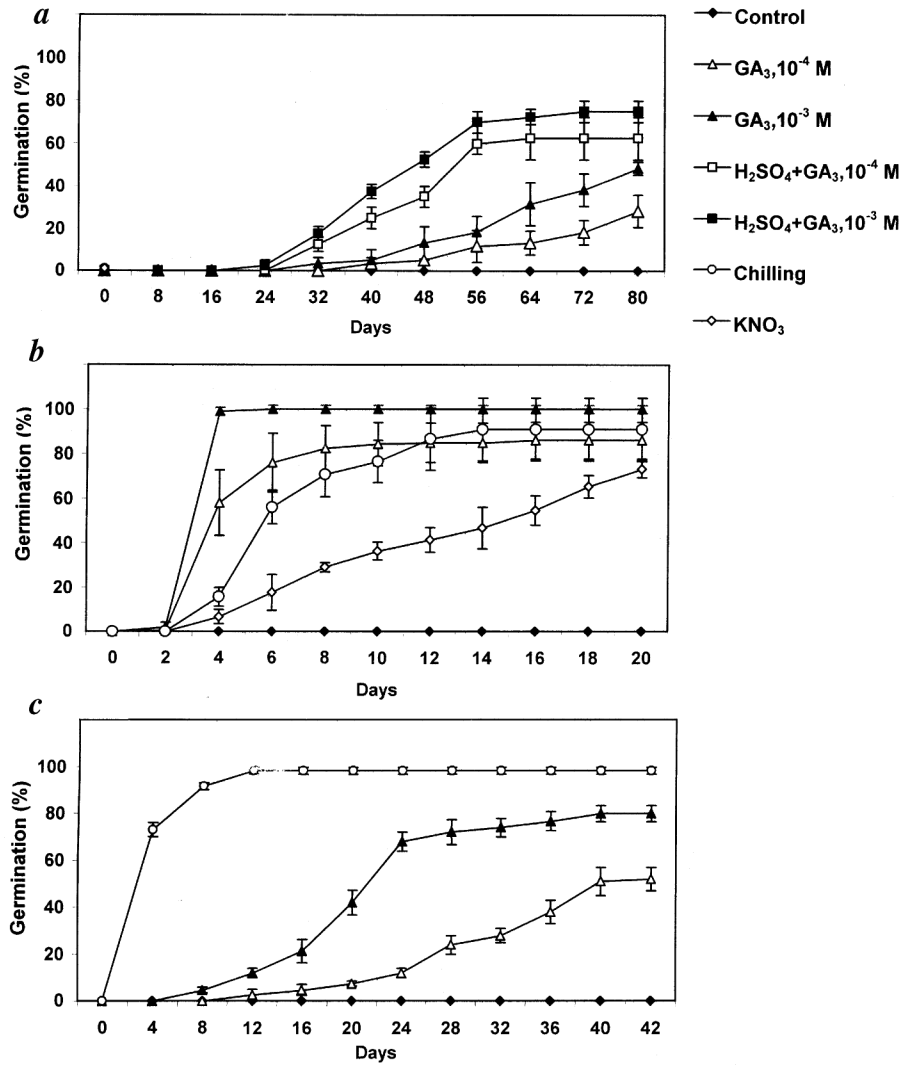

Figure 1. Time course of seed germination of *Podophyllum hexandrum* (***a***), *Hyoscyamus niger* (***b***) and *Inula racemosa* (***c***) as affected by different treatments. $n = 3 \pm$ SD.

to the cold deserts of the Lahaul and Spiti district. Seeds of *P. hexandrum* were extracted by depulping the ripened berries followed by thorough washing with tap water, whereas those of the other species were separated manually. The seeds were air-dried for a fortnight at room temperature, after which they were stored in polyethylene bottles at room temperature for studies described here.

Seed germination was assessed as early as possible following harvest in all cases, except *R. australe* where eight-month-old seeds were tested. Uniform (as far as possible) seeds were surface-sterilized with 0.1% aqueous solution of mercuric chloride for about 5 min. Thereafter, they were washed thoroughly with tap water and kept submerged in distilled water for 24 h at 25 ± 2°C. The seeds were transferred to petri plates lined with three layers of filter paper made wet with distilled water and allowed to germinate in an incubator at 25 ± 2°C under continuous illumination provided by fluorescent white light (PAR: 40 μmol m^{-2} s^{-1}). Seed germination was recorded at periodic intervals, the radicle emergence (2–5 mm) serving as an index of germination. Fifty seeds in triplicate were used for each treatment.

Prior to germination assays, the seeds of different species were subjected to various physico-chemical and hormonal treatments as follows: Stratification – the surface-sterilized seeds soaked in distilled water for 24 h were subjected to low temperature (2–4°C) treatment for 1 month in all species, except *B. persicum* where a two month treatment was given. Acid scarification – seeds were treated with concentrated H_2SO_4 for varying durations depending upon the species followed by thorough washing. Thus, 30 s (*B. persicum*, *C. carvi*), 1 min (*H. niger*, *I. racemosa*) and 8 min (*P. hexandrum*) treatments were given; these treatment durations were worked out on the basis of trial experiments. Potassium nitrate (KNO_3) – surface-sterilized seeds were soaked in 0.2% aqueous solution of KNO_3 for 24 h

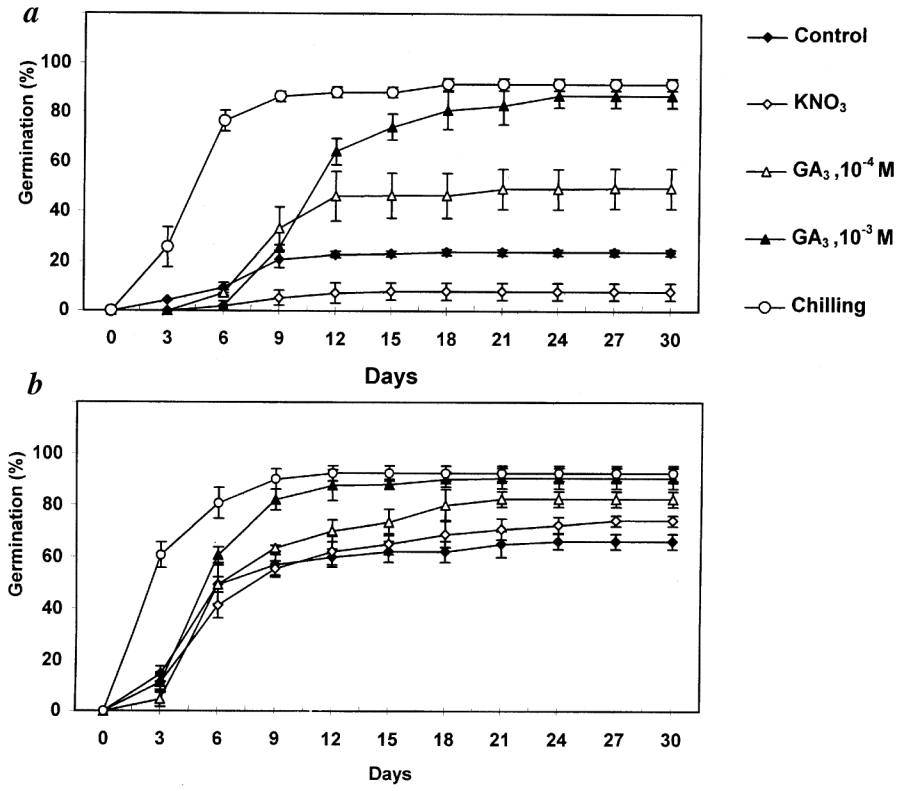

Figure 2. Time course of seed germination of *Carum carvi* (***a***) and *Saussurea costus* (***b***) as affected by different treatments. $n = 3 \pm$ SD.

followed by germination on substratum moistened with 0.2% KNO_3. Gibberellic acid (GA_3) – surface-sterilized seeds were kept submerged in aqueous solution of GA_3 for 24 h followed by germination on substratum moistened with GA_3 solution. Combined treatment with H_2SO_4 and GA_3 (H_2SO_4/GA_3) – In case of *P. hexandrum*, seeds were treated with H_2SO_4 for 8 min followed by thorough washing, soaking with GA_3 solution for 24 h and subsequent germination on substratum moistened with GA_3 solution.

The presence of any chemical inhibitors of germination in dormant seeds was detected by monitoring the effect of dormant seed extracts on germination of nondormant seeds of *Triticum aestivum* L. and *Brassica juncea* (L.) Czern. The seed extracts were prepared from 50–300 seeds per 50 ml water depending upon the seed size in different species (*P. hexandrum* – 50 seeds; *H. niger* – 300 seeds; *I. racemosa*, *B. persicum*, *C. carvi* – 200 seeds). Seeds of the two test species were soaked in the seed extracts before germinating, as described earlier. Seedlings of medicinal plants resulting from the seeds germinated through various treatments were transferred to pots for further growth in a naturally lit glasshouse.

The status of seed dormancy and germination of seven selected medicinal plants from Lahaul and Spiti is shown in Table 2. Fresh seeds were tested; as an exception, the seeds of *R. australe* were eight-month-old. The relative effectiveness of different physico-chemical and hormonal treatments in causing dormancy removal and germination improvement in different species is also summarized in the Table 2. The specific patterns of seed response to different treatments in *P. hexandrum*, *H. niger*, *I. recemosa*, *C. carvi* and *S. costus* are shown in Figures 1 and 2.

B. juncea showed 86% germination after two days in control. Extracts of *B. persicum* and *C. carvi* seeds inhibited germination by 48 and 28%. Similar results were also obtained with *T. aestivum* seed germination. However, the inhibitory effect of dormant seed extracts was almost completely lost after three days (data not shown). These observations indicate the presence of chemical inhibitors in dormant seeds of *B. persicum* and *C. carvi*; the inhibitors are apparently metabolized to non-inhibitory products by the germinating seeds of test species. The seed extracts of *P. hexandrum*, *I. racemosa* and *H. niger* had no effect on germination of either test species (data not shown).

The survival rate of seedlings transplanted from petri dishes to pots in the glasshouse was high in *P. hexandrum*, *S. costus*, *I. racemosa*, *C. carvi* and *R. australe* and low in *H. niger* (Table 2). Ten-month-old plants of these species are shown in Figure 3. The transplanted seedlings of *B. persicum* did not survive beyond four weeks.

The responses of seeds to different treatments were strongly species-specific. It is obvious from the present

data and similar work reported by other authors that the responses of dormant seeds of the same species to different factors are variable, sometimes even qualitatively, depending upon the habitat/location of collection, duration of storage, etc. This aspect, central to the objective of the present study, will be highlighted by discussing the case of *P. hexandrum*. Seeds of this endangered species from alpine regions have been reported to exhibit greater germination[11] than those from the subalpine regions[12]. Badhwar and Sharma[13] reported 44% germination upon sowing *P. hexandrum* seeds with fruit pulp. This response, however, was lacking in the study of Nautiyal *et al.*[14]. Likewise, the effect of GA_3 at dormancy removal has been reported to be highly inconsistent. Whereas GA_3 treatment promoted germination by a factor of two when the fruits (berries) were treated, there was no effect altogether when seeds removed from the berries were treated. The ineffectiveness of GA_3 was also reported[15]. Nadeem *et al.*[16] reported a marked promotion by GA_3 in seeds stored for 5 months at 4°C, but not in freshly harvested seeds. This is apparently attributable to the storage-dependent changes in seed sensitivity towards GA_3. In the present study, GA_3 strongly relieved the seeds from dormancy; GA_3 effect was further enhanced when seeds had been acid-scarified prior to applying the hormone (Figure 1 *a*). Apparently, seed coat hardness seems to be involved in dormancy besides a requirement for after-ripening. During the after-ripening of seeds, GA_3 synthesis might be stimulated to an extent necessary for dormancy removal.

The foregoing account indicates that different populations of the same species vary as far as the requirements for seed dormancy removal are concerned. Obviously, a population-specific characterization of seed behaviour would be essentially crucial for *ex situ* conservation and commercial cultivation. Thus, the present data could have implications for conservation and cultivation of the studied populations.

Similar to our observations, effectiveness of low temperature in causing dormancy removal has also been reported in other populations of *C. carvi*[17] and *B. persicum*[18]. The low temperature requirement appeared to be replaced by GA_3 in *I. racemosa* and *C. carvi*, but not in *B. persicum*, further signifying the species specificity in responses even of closely related species. Low-temperature treatment of seeds could be easily adopted for the cultivation of species wherever it proved effective.

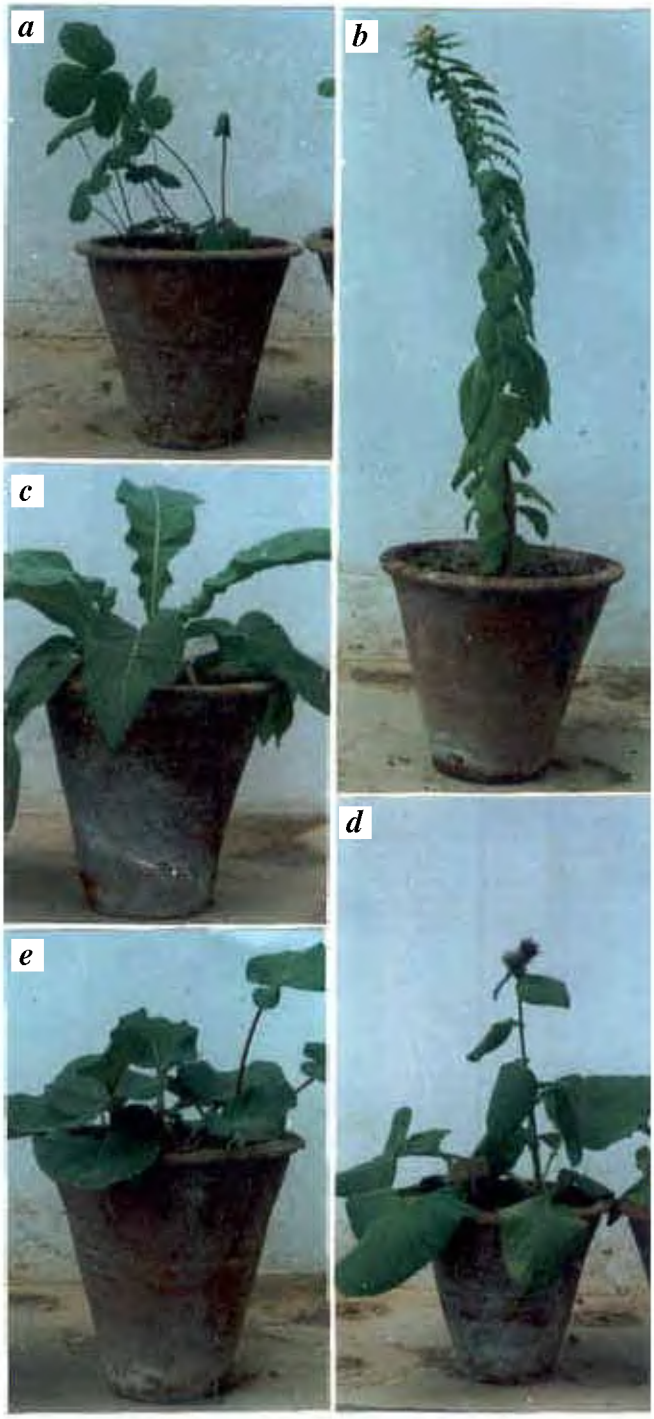

Figure 3. Ten-month-old plants of *P. hexandrum* (***a***), *H. niger* (***b***), *I. racemosa* (***c***), *S. costus* (***d***) and *R. australe* (***e***) growing under glasshouse conditions. Seed-derived seedlings were transferred from petri dishes to pots for further growth.

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RAPD analysis reveals the presence of mainland Indian and Indonesian forms of *Erianthus arundinaceus* (Retz.) Jeswiet in the Andaman–Nicobar Islands

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Three new accessions of *Erianthus arundinaceus* (Retz.) Jeswiet a wild relative of sugarcane, collected recently from the Andaman–Nicobar group of islands were characterized using RAPD markers, in relation to two distinct forms of the species from mainland India and Indonesia. Two of the clones from the Middle Andaman showed molecular profiles similar to the mainland Indian forms while the third clone from the Katchal Island of the Nicobar group showed profiles similar to the Indonesian forms. The study clearly establishes the presence of both mainland Indian and Indonesian forms of *Erianthus arundinaceus* in the Andaman–Nicobar group of islands. The distribution of the two forms was found to be in accordance with the geographical proximity of the collection sites with respect to the Indian subcontinent or the Indonesian Archipelago.

Keywords: Andaman–Nicobar, *Erianthus arundinaceus*, RAPD markers.

ERIANTHUS is a close relative of sugarcane (*Saccharum officinarum* L.) and an important member of the *Saccharum* complex[1]. There are several species of *Erianthus*, majority of which are found in the Indian subcontinent, including *E. arundinaceus*, *E. procerus*, *E. longisetosus*, *E. bengalense*, *E. ravennae*, *E. fulvus*, *E. elephantinus* and *E. hookeri*. This genus is believed to have originated in the Indo–Myanmar–China region, spreading subsequently to the adjoining areas[1]. *E. arundinaceus* is widely distributed in India, China, Myanmar, Thailand, Philippines, Indonesia and New Guinea.

The species that is considered most important among *Erianthus* for exploitation in sugarcane breeding is *E. arundinaceus* (Retz.) Jeswiet represented by cane-forming types, with tremendous ability for biomass production and a high level of tolerance to biotic and abiotic stresses. This species is extensively distributed in the Indian subcontinent as well as in most of the South East Asian countries and is considered to be an important source for breeding improved sugarcane varieties with high yielding ability, tolerance to environmental stress and resistance to diseases and pests. Serious efforts are underway to utilize *E. arundinaceus* in sugarcane breeding programmes in several countries including India. The major part of the *E. arundinaceus* germplasm available today is either from the Indian subcontinent or from the Indonesia–Papua New Guinea region, which form the major areas of diversity for the species. In view of its importance in sugarcane breeding, considerable attention had been given for the collection and characterization of the species[2].

Serious efforts to collect the wild germplasm of sugarcane in India began in 1933. Several *Erianthus* clones were collected from the distributional areas in the country during the explorations conducted over the years[3–6]. Consequently a large and well-characterized *E. arundinaceus* germplasm is available today for exploitation[2]. Though the existing collections represent most of the geographical diversity available in the country, representation from the Andaman–Nicobar group of islands was lacking till recently. The Andaman–Nicobar group of islands are positioned between the Indian subcontinent, the Malay Peninsula

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