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Seed longevity, germination and seedling vigour of *Rheum australe* D. Don: *A* step towards conservation and cultivation

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ABSTRACT

A 60 months study was carried out to assess the seed longevity and effect of duration of storage period on viability, germination and seedling vigour in *Rheum australe* D. Don. (rubarb), a highly valued medicinal herb. The species is in high demand and has endangered status therefore, required conservation/cultivation interventions. The study was carried out using seeds of same lot after storage of every six months (0, 6, 12, 18 and so on) till complete loss of seed viability. Freshly harvested seeds exhibited high seed viability (94%) and were non-dormant; above 80% germination occurred within a week. The above status was completely retained till 12 months storage. Beyond this period both seed viability and germination declined consistently with the progression of storage period. A complete loss of viability was observed beyond 60 months storage. Seed pre-treatments tested namely, GA_3, KNO_3 and chilling neither affected significantly the final germination of fresh seeds (86, 80 and 78%, respectively) nor differentially stored seeds. However, GA_3 (1 mM) and chilling significantly reduced the time required for germination. The seed longevity (time taken for 50% decline in seed viability) of *R. australe* was about 45 months, but a storage period of no longer than 24 months can be recommended. During 24 months storage period the seed viability and germination were reasonably high and resulted in the production of healthy seedlings with high survival rate. The outcome was favourable for the conservation/cultivation of the species.

1. Introduction

Rheum australe D. Don. (Polygonaceae), commonly known as Himalayan rhubarb is an valuable and important medicinal herb, traditionally used to cure a wide range of ailments, including blood disorder, bronchitis, constipation, fractured bone, gastric, menstrual disorder, piles, rheumatism, sprains, ulcer and wounds (Chauhan, 1999; Rokayaa et al., 2012). Various pharmacological experiments have convincingly confirmed its anticancer (Rajkumar et al., 2011), antidiabetic (Radhika et al., 2010), antimicrobial (Babu et al., 2003), anti-inflammatory (Chauhan et al., 1992), antioxidant (Krenn et al., 2003), hepatoprotective (Akhtar et al., 2009), immuno-enhancing (Kounsar et al., 2011) and nephroprotective (Alam et al., 2005) activities, showing the rational behind its traditional uses.

The plant is endemic to Himalayan region and grows in alpine and sub alpine zone at an altitude of 3300–5200 m asl (Rokayaa et al., 2012). The species is in huge demand due to its remarkable medicinal properties, which led to illegal over-exploitation of natural habitats, resulting in rapid decline in wild population, where it has become rare (Nautiyal et al., 2003). According to IUCN criteria the plant has endangered status and is banned for trade unless or until accompanied by either a 'cultivation certificate' or 'legal procurement certificate' from the designated forest authorities (Ved et al., 2003).

Keeping in view, the endangered status, medicinal importance and ever-increasing demand, *R. australe* is included in the list of important species that are identified as top-priority species for conservation and cultivation (Nautiyal et al., 2003). For such purpose, the information regarding seed germination and longevity might be of immense help. There is a bulk of information regarding its taxonomy, distribution, medicinal uses and analysis of active compound (Rokayaa et al., 2012 and references therein). However, there are only few reports concerning seed germination and seedling growth (e.g. Nautiyal et al., 2003; Sharma et al., 2006; Kandari et al., 2012; Rokayaa and Münzbergova, 2012) and the information regarding seed longevity and effect of long-term ambient storage on seed viability, germination and seedling growth is still lacking. Seed following harvest undergo numerous metabolic changes that are

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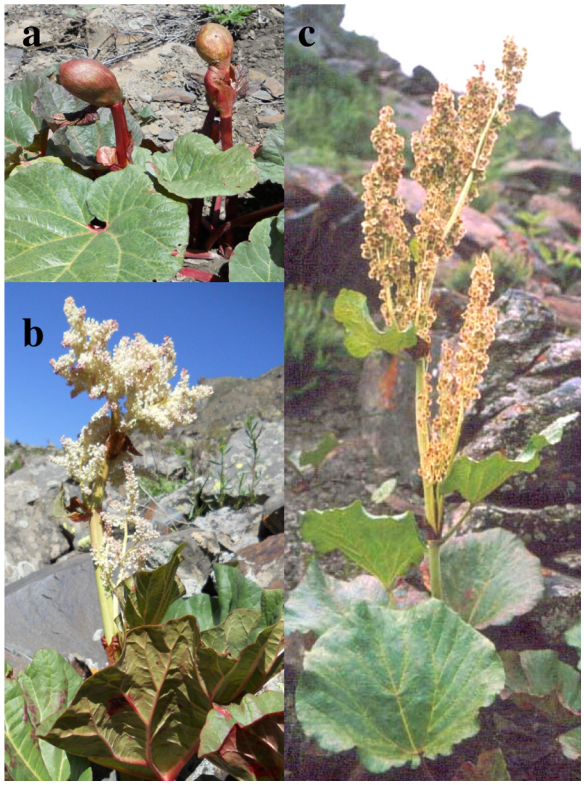

Fig. 1. Different stages of plant growth (a: vegetative, b: flowering and c: fruiting) in *R. australe* at its natural habitat in Lahaul, Himachal Pradesh, India.

eventually reflected in altered status of viability and germination. Such storage-dependent changes are strictly species-specific and their understanding has definite implications for conservation and cultivation of the species. Therefore, we report here the seed viability, germination and seedling growth performance of *R. australe* in relation to long-term storage (60 months).

2. Material and methods

2.1. Plant material

The mature seeds of *R. australe* were collected from a wild population (Fig. 1) in Pattan valley (latitude: 32°38′36″ N, longitude: 76°55′30″ E and altitude: 3900 m above mean sea level), Lahaul, Himachal Pradesh, India during August-September 2003. The seeds were air-dried in shade and stored in airtight container at room temperature (average: 15 ± 3 °C) for subsequent studies.

2.2. Seed viability test

Seed viability was determined through TTC (2,3,5-triphenyl tetrazolium chloride) reduction test shortly after collection and at regular intervals during the subsequent storage period. The surface sterilized seeds were imbibed in distilled water for 24 h at 25 ± 1 °C. Thereafter, the embryos were excised from the seeds and incubated with 0.1% TTC solution in dark for 24 h and examined for staining intensity/pattern. Seeds with completely stained embryo were considered viable.

2.3. Seed germination assays

Seeds were surface sterilized with 0.1% $HgCl_2$ for 5 min, washed thoroughly under tap water and soaked in distilled water for 24 h at 25 ± 1 °C. Thereafter, the seeds (25, in triplicate) were transferred to Petri dishes lined with three layers of filter paper moistened with distilled water and allowed to germinate in a seed germinator at 25 ± 1 °C under continuous illumination provided by the fluorescent white light (PAR: 40 μmol m^{-2} s^{-1}). Seeds were considered germinated upon radicle emergence (≥2 mm); germinated seeds were counted periodically. The mean germination time (MGT) was calculated as follows (Hartmann et al., 1989): MGT = Σ (nd)/N, where, n = number of seeds germinated after each incubation period in days, d and N = total number of seeds emerged at the end of the test. Seed longevity period was determined as the time taken for 50% decline in seed viability (Ellis et al., 1990).

2.4. Seed pre-treatments

2.4.1. Cold stratification

Surface-sterilized seeds soaked in distilled water for 24 h were transferred to moist filter papers and subjected to low temperature (4 °C) for 30 d after which they were allowed to germinate at 25 °C.

2.4.2. KNO_3 and GA_3 treatments

Surface-sterilized seeds were soaked in aqueous solution of 0.2% potassium nitrate (KNO_3), 0.1 and 1.0 mM gibberellic acid (GA_3) for 24 h followed by germination on moist filter paper at 25 °C.

2.5. Seedling growth and survival

The germinated seeds from the differentially stored seeds were allowed to grow in Petri plates to an appropriate size and examined the number of healthy seedlings. Thereafter, the seedlings were transferred to pots in glasshouse for further growth and survival rate. The number of healthy seedlings and survival rate was calculated as following:

$$\text{Healthy seedlings}(\%) = \text{Number of healthy seedlings} / \text{Total number of seeds germinated} \times 100$$

$$\text{Survival rate}(\%) = \text{Number of seedling survived} / \text{total number of seedlings sown} \times 100$$

2.6. Data analysis

All experiments were carried out in triplicate. Data are presented as arithmetic mean ± standard deviation. The significance of difference between control and effectors (chilling/GA_3/KNO_3) treated or differentially stored seeds was assessed based on the Student's *t*-test.

3. Results

3.1. Salient features of seeds

The seeds are reddish purple, angled with narrow wings and notched apex. To determine the seed size and weight, 25 dry seeds were used. The seed size was 0.72 ± 0.07 × 0.62 ± 0.04 cm (length × width) and the average seed weight of dry and 24 h soaked (beyond 24 h soaking the seed weight remain constant) seeds was 13.1 ± 0.9 and 24.9 ± 1.5 mg, respectively. Thus, a 90% increase in seed weight was observed due to soaking.