Breaking seed dormancy in *Hippophae salicifolia*, a high value medicinal plant

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Abstract Seed dormancy is an important limiting factor in exploitation of an economically important species to its fullest. *Hippophae salicifolia* D. Don (seabuckthorn), a rich source of medicinal metabolites shows both exogenous and endogenous dormancy. Evidently, we recorded a high seed viability (94 %) but poor germination (22 %) of untreated seeds. We applied different pre-sowing seed priming treatments including NaCl (50, 100, 200, 500 mM), KNO₃ (0.1, 0.2, 0.3 %), Thiourea (1, 2, 3 %), GA₃ (100, 250, 500 mg/L), Sulphuric acid (98 %) and cold (4 °C) and warm water (65 °C) stratifications to explore improvements in its germination percentage, if any. We found KNO₃ (0.1 %) and Thiourea (1 %) treatments to be superior to other methods for enhancement of mean seed germination percentage of *H. salicifolia*. Considering the practical applicability and cost effectiveness, these treatments can be applied to overcome seed dormancy and recommended for mass multiplication through seeds of *H. salicifolia*.

Keywords Seabuckthorn · *Hippophae salicifolia* D. Don · Pre-germination treatment · Exogenous dormancy · Endogenous dormancy

Introduction

Hippophae salicifolia D. Don is a lesser explored high value medicinal species of seabuckthorn (Family Elaeagnaceae).

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It generally grows as a medium to tall tree (4–7 m) at 1,500–3,200 m asl (Singh et al. 2008). Compared to *H. rhamnoides*, it is only mild thorny and contains all essential polyunsaturated fatty acids particularly omega-3 and omega-6, high quality berries with late maturation character, etc. Vitamin C content in *H. salicifolia* is highest among all species of seabuckthorn (Gupta and Ahmed 2010). In addition to its high medicinal and nutraceutical properties, seabuckthorn is also valued for its high ecological roles in nature (Gupta and Ahmed 2010; Jain et al. 2010).

H. salicifolia generally propagates by root suckers, softwood and hardwood cuttings and seeds (Singh et al. 2008). Plants are monoecious and both male and female seeds are produced in equal and large quantities. Seeds remain viable for two years but freshly harvested seeds suffer with physiological dormancy (Singh et al. 2008). In nature, cold treatment of seabuckthorn seeds is essential to overcome the dormancy. Such conditions are provided naturally by the habitat in which the plant is growing. Artificially, pre-germination physiochemical treatments can improve seed germination rate, performance and result into faster and synchronized seed germination (Gupta 2003). The common seed-priming agents used for faster seed germination include concentrated H₂SO₄, KNO₃, GA₃, PEG, thiourea and some of them have been applied in *H. rhamnoides* as well with variable degrees of success (Sankhyan et al. 2005; Olmez 2011). Improvement of seed germination percentage in *H. salicifolia* has received relatively lesser attention despite its high medicinal value. Thus, with an aim to develop rapid methods of cultivating the plant while ensuring its sustainable utilization, we have applied various pre-germination treatments for fast and improved germination rates in the temperature range 20–30 °C.

Materials and methods

Mature healthy seeds of *H. salicifolia* were collected during the first week of November 2010 from Defence Institute of Bio-Energy Research (DIBER) Field Station at Auli, India. Immediately after collection, seeds were cleaned manually, dried for one week in sunlight and stored at 25 °C until use. Seed viability was determined using Tetrazolium chloride (TTC) method, as described by Kumari and Dahiya (2007). Seeds were disinfected by immersing in 0.5 % sodium hypochlorite solution for 2 min followed by rinsing thoroughly with distilled water four times. The sterilized seeds were soaked for 48 h in different concentration of NaCl (50, 100, 200, 500 mM), KNO₃ (0.1, 0.2, 0.3 %), thiourea (1, 2, 3 %) and GA₃ (100, 250, 500 mg/L) solutions at 25 °C. For sulphuric acid treatment, seeds were placed in separate beakers containing sulphuric acid (98 %) and stirred for 1, 2 and 5 min to get uniform effect. For cold and hot water stratification, seeds were kept at 4 °C and 65 °C, respectively for 24, 48 and 72 h. Treated seeds were washed thoroughly with distilled water and placed in Petri plates containing moistened Whatman filter paper. Petri plates were kept at 25±0.5 °C in growth chamber and moistened as needed with distilled water. A set of seeds without pre-sowing treatments were considered as control. Seed germination was recognized by emergence of radical, while whole experiment was monitored up to 90 days.

Result and discussion

For commercial cultivation and utilization of seabuckthorn, it is imperative to propagate using best material that has been selected primarily for the fruit yield. Coupled with seed dormancy, *H. salicifolia* is especially more difficult to propagate as it is reported to have lowest germination rate among the Indian gene pool of *H. rhamnoides*, *H. tibetana* and *H. salicifolia* (Singh 2009). We here present a simple and inexpensive method to boost the plantation of salicifolia seabuckthorn that can easily be applied by small scale breeders and cultivators.

The viability of fresh seeds of *H. salicifolia* was estimated to be 94 %. However, as seen in the control seeds, the native seed germination percentage stood at 22 %, even lesser than reported earlier by Singh (2009) for *H. salicifolia*. Clearly, the failure of germination is attributed to seed dormancy. Even though, physiological dormancy of seabuckthorn seeds (endogenous) is well known (Singh et al. 2008; Dwivedi et al. 2009), members of the family Elaeagnaceae are also reported to show exogenous dormancy as fruits have stony endocarp (Baskin and Baskin 1998). Thus, the methods employed here were classified into two categories based on the nature of dormancy they targeted. In general, reasonable success was obtained in all but one method, but breaking the seed coat dormancy clearly had more profound effects compared to breaking the embryo level physiological dormancy.

Fig. 1 Germination percentage of *Hippophae salicifolia* seeds as influenced by various pre-sowing seed priming treatments including NaCl (50, 100, 200, 500 mM), KNO₃ (0.1, 0.2, 0.3 %), thiourea (1, 2, 3 %), GA₃ (100, 250, 500 mg/L), sulphuric acid (98 % for 1, 2 and 5 min) and cold (4 °C) and hot water (65 °C) stratifications (24, 48, 72 h). A set of seeds without pre-sowing treatments was considered as control (C)

Breaking exogenous seed dormancy

Scarification achieved by mechanical, chemical or thermal treatments primarily breaks exogenous dormancy by targeting physical alterations of the seed coats (Kumari and Dahiya 2007). Chemical methods employed for this purpose may generally include high ionic strength (i.e., salts), extreme pH (generally acids) or organic solvents. Salts in general, induce osmotic pressures by sticking to cell membranes and cell walls, thereby affecting the water potential of the cytosol and cellular extensibility. This, in turn affects seed germination and seed growth (Tobe et al. 2004). Among the salts, sodium chloride (NaCl) and potassium nitrate (KNO₃) are the two well-studied seed priming treatments (Yücel and Yilmaz 2009; Ramzan et al. 2010). While we found an improvement of nearly three times in germination percentage using NaCl pre-treatment, KNO₃ improved seed germination by four times or even higher (Fig. 1). A peak germination rate of 96 % was achieved using 0.1 % KNO₃ pre-treatment, but higher concentrations of KNO₃ were found inversely related to mean seed germination. Improved seed germination post KNO₃ treatment, in general, is in agreement with earlier reports (Yücel and Yilmaz 2009; Ramzan et al. 2010). Pre-treatment with NaCl improves seed germination with peak success rate of 69 % germination on treatment with 200 mM NaCl (Fig. 1). Ertan and Ismail (2006) reported an identical NaCl concentration as the best to improve germination and vigour of pepper seeds.

Effect of thiourea on seed germination was comparable to KNO₃. Thiourea (1.0 %) resulted in 89 % seed germination but higher concentrations reduced seed germination significantly (Fig. 1). Similar observations have also been made earlier by Pandey et al. (2000) in *Aconitum* spp. In contrast to the other treatments, H₂SO₄ proved to be a germination inhibitor, as small duration exposure to it also adversely affected germination rates. Olmez et al. (2004) considered it to be an inhibitor even at low concentrations.

Breaking endogenous seed dormancy

Water stratifications are generally employed to overcome dormancy at the embryo level (Olmez 2011). In case of seabuckthorn, seeds are conventionally soaked in moist sand for 25 days prior to sowing in the month of May at higher altitudes in India (Dwivedi et al. 2009). They can also be sown directly in nursery beds in the month of November when cold treatment is naturally provided before germination (Dwivedi et al. 2009). In nature, this works well for seabuckthorn and germination percentages as high as 85 have been observed (Dwivedi et al. 2009). However, in our hands, when seeds were exposed to cold water

stratifications, maximum mean seed germination observed was 52 % (Fig. 1). Warm water stratifications, similarly had average success rate of 48 % germination.

Physiological dormancy is regulated in seeds due to relative levels of growth promoters (GA₃) and inhibitors (Abscissic acid, ABA). For germination to take place, level of GA₃ should exceed the level of ABA. Stratification by cold or warm water changes the endogenous levels of these phytohormones thereby breaking the seed dormancy. However, as we did not see improvement in germination percentage of seabuckthorn seeds to the same degree as observed by breaking the exogenous dormancy, we soaked the seeds directly in different GA₃ concentrations. GA₃ proved third best pre-treatment agent in the present study (Fig. 1). Thus, in seabuckthorn, endogenous dormancy is more effectively broken by GA₃ than stratification treatments.

Concluding statements

The pre-germination treatments are primarily meant to cause partial seed hydration initiating pre-germinative metabolic processes ahead of radical protrusion. The resulting enhanced rate of cell division and metabolic activities in primed seed lead to altered germination patterns (Taylor and Harman 1990). Here, the application of chemical scarification has apparently made the seed coat permeable to water and gases. Introducing the physical alterations in seed coat alone was found to enhance the seed germination by 3–4 fold. However, when alterations were targeted in embryo only, improvement in seed germination percentage was still two fold. From practical point of view, adopting the pre-treatment of seabuckthorn seeds with salts like KNO₃ (0.1 %), thiourea (1 %) or even NaCl (200 mM) is an effective and faster method compared to existing practice of moist sand treatment. As the germination percentage is far higher, the pre-germination treatments are also recommended for their cost effectiveness in *H. salicifolia*.

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