



In vitro propagation of male and female *Hippophae salicifolia* D. Don

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Abstract

Hippophae salicifolia D. Don. is a deciduous, temperate, and dioecious tree of great economic and ecological importance. In this study, an *in vitro* propagation protocol was established for male and female explants of *H. salicifolia*. The most suitable explants were selected by screening the various types of explants such as dormant and active axillary buds, apical buds, nodal parts, and leaves. The explants were selected every month throughout the year to test the response, and only dormant and active buds were able to generate a response. Accumulation of phenolics was one of the most significant problems in the *in vitro* propagation of *H. salicifolia*, which was overcome by using an antioxidant solution of 1500 mg L⁻¹ each ascorbic acid and citric acid followed by a lower temperature treatment, and pre-culture in 1.2% (w/v) agar supplemented with 0.01% (w/v) myo-inositol and 3% (w/v) sucrose. Active buds proved to be the best explant type to propagate *H. salicifolia in vitro*, either on Murashige and Skoog (MS) or Lloyd and McCown Woody Plant media supplemented with high cytokinin concentrations. Half- and full-strength MS media were used for rooting the *in vitro*-grown shoots, and the rooting percentage was higher in the half-strength MS media than in the full strength. There were no significant differences in the responses of male and female explants in various culture conditions. Female plants showed better acclimatization than male plants.

Keywords Dioecious · Dormant buds · Active buds · Phenolic accumulation · Woody plant medium

Introduction

Hippophae (sea buckthorn) is among the genera of plants that are valuable due to their multifarious benefits. *Hippophae* species are used in *materia medica* of several traditional medicinal systems (Suryakumar and Gupta 2011), due to the extraordinary nutritional value and medicinal importance (Acharya et al. 2010). The genus *Hippophae* belongs to the family Elaeagnaceae and order Daphnales, commonly known as sea buckthorn and Leh berry in India. *Hippophae salicifolia* D. Don. is distributed in higher elevations of Indian Himalaya, Himachal Pradesh, Uttarakhand, Sikkim, and Arunachal Pradesh (Chauhan 1999). In Uttarakhand Himalaya, *H. salicifolia*, commonly known as Amesh or Chuk, is

distributed in Uttarkashi, Rudrapur, Chamoli districts of the Garhwal region, and Pithoragarh district of the Kumaon region. The local inhabitants use the plant as chutney, juice, a substitute for tomatoes, fuelwood, and fencing, and sometimes for veterinary and medicinal purposes in regions of its natural habitat (Dhyani et al. 2010). Vitamin C (ascorbic acid) is present as a nutrient of primary importance in *Hippophae* sp., which is reported at the highest level in *H. salicifolia* among all the sea buckthorn species (Singh 2009). The juice of this species is also rich in carotenoids, flavonols, vitamin E, and minerals such as Zn, Cu, Fe, Ca, Mg, Na, and K (Singh 2009). Both seed and pulp oil of *H. salicifolia* are rich in carotenoids; tocopherols, sterols, and saturated and unsaturated fatty acids involved in the essential fatty acids, and in alpha-linolenic acid, and linoleic acid, and conditionally essential fatty acids such as gamma-linolenic acid (Ranjith et al. 2006; Singh and Gupta 2015).

Sea buckthorn, despite being a common and a widespread species, deserves conservation measures because its distribution pattern has been described as “highly fragmented” and genetically diverse (Dwivedi et al. 2009). According to Small et al. (1992), in Hungary, wild sea buckthorn is rarely

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