Optimization of in vitro regeneration of *Haloxylon salicornicum*: a keystone species of extreme arid regions

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Abstract *Haloxylon salicornicum* is an economically important perennial woody shrub of family Amaranthaceae and is a good source of food additives, fodder, fuel and nutrients in Indian Thar Desert. In vitro regeneration of *H. salicornicum* through nodal shoot segments is achieved in present investigation. Young green shoots in horizontal orientation were found to be more responsive than vertical orientation. Axillary shoot buds were activated on MS medium containing 10 μM BAP. Adventitious roots with intense root hairs were induced on horizontally placed shoots on half strength MS medium containing 2 μM NOA + 100 mg L^{-1} activated charcoal. This is a first report on micropropagation of *H. salicornicum*. This protocol can be a useful means for in vitro characterization, mass propagation and conservation of the plant for future prospects.

Keywords Arid environment · Haloxeric · *Haloxylon salicornicum* · Micropropagation · Underutilized

Introduction

Establishment of cell, tissues and organ cultures and regeneration of plantlets in vitro have many avenues in plant biotechnology (Dagla 2012). An in vitro culture technique offers advantage to clone large number of plantlets in relatively limited time and space regardless of

seasonal barrier. In vitro culture of plant cells and their organs forms the basis of practical applications in agriculture, horticulture and industries. It also helps in screening and selection of the drought and salt tolerant ability of cells/plants (Upendra and Dagla 2016). Molecular characterization, conservation and mass propagation of native plants of Indian Thar Desert are needed because of their economic importance in the region (Dagla and Shekhawat 2005a, b, 2006; Dagla et al. 2007, 2012, 2014; Gehlot et al. 2014; Goswami and Dagla 2017; Upendra et al. 2017; Nair et al. 2018). Plants of Indian Thar Desert are sources of food supplement, feed, fuel, and nutrients for local people of the area and even source of gene(s) for future plant breeding programme. In vitro multiplication of *Haloxylon recurvum* and *Leptadenia pyrotechnica* from Indian Thar Desert has been reported by Dagla and Shekhawat (2005b) and Nair and Dagla (2016). Establishment of in vitro cultures and mass multiplication from mature explants of these plants are still a big challenge. *Haloxylon salicornicum* (Moq.) Bung belongs to the family Amaranthaceae and known as a Lana in the region. It is an underutilized leafless woody perennial shrub and is a good source of food additives, fodder, fuel and nutrients (Fig. 1a). Mature woody stems of this plant have high fuel efficiency and burn with little smoke. Ash of this plant is used for cleansing of teeth and young green branches are good source of fodder for camel. Mixed flour of the seeds of *H. salicornicum* and pearl millet (*Pennisetum glaucum*) is used for preparation of special dish 'Dhokla' in the region. The plant is an efficient soil binder and stabilizes shifting sand dunes and enhances soil fertility (Dagla and Shekhawat 2006). Plant is also used as a source of compounds with antituberculosis (Bibi et al. 2010), anti-inflammatory (Verma et al. 2014) activities and spontaneous anti contractions of uterus (Saleem et al. 2013). There was

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Fig. 1 **a** *Haloxylon salicornicum* in natural habitat; activation of axillary shoots on MS medium containing, **b** 1 μ M BAP, **c** 5 μ M BAP, **d** 10 μ M BAP, **e** 15 μ M BAP, **f** 20 μ M BAP

a need to develop protocol for cloning of *H. salicornicum* by vegetative means for large scale plantation in the desert areas. Preliminary efforts on establishment of in vitro culture using mature nodal shoot segments for cloning of *H. salicornicum* failed due to hindrances in surface sterilization and poor responses of mature explants. Adaptive modifications of these plants make them more recalcitrant for establishment of in vitro cultures using mature explants.

Growth phase of explants and its orientation on culture medium is standardized in present investigation for in vitro mass multiplication of *H. salicornicum*. This is a first report on in vitro multiplication *H. salicornicum* using nodal shoot segments of mature plants. The present study can be a useful means for in vitro characterization, mass propagation and conservation of *H. salicornicum*.

Fig. 2 Activation of axillary shoots from nodal shoot segments on MS medium containing 10 μM BAP: **a** axillary shoot number, **b** axillary shoot length; repeated sub-culturing of mother explants: **c** axillary shoot number **d** axillary shoot length. The observed data

were analyzed by one way-ANOVA and Duncan's multiple range test ($P < 0.05$) and each value represents an average number of three replicates using 25 explants. Different letters represents statistically significant differences with standard deviation error bars

Materials and methods

Selection and surface sterilization of explants

Mature woody and young green shoots of *H. salicornicum* were treated with 70% alcohol for 30–40 s. These were then surface sterilized with 0.1% (w/v) aqueous solution of HgCl_2 for 4–5 min and rinsed 3–4 times with autoclaved distilled water.

Activation and mass multiplication of axillary shoots

Surface sterilized explants were inoculated horizontally and vertically on semi-solid MS (Murashige and Skoog 1962) medium containing 3% (w/v) sucrose and 0.8% (w/v) agar supplemented with various concentrations (0, 1, 5, 10, 15, 20 μM) of 6-benzylaminopurine (BAP) and kinetin. Cultures were maintained at $28 \pm 2^\circ\text{C}$ under $35\text{--}40 \mu\text{mol m}^{-2} \text{s}^{-1}$ irradiance and 12 h d^{-1} photoperiod

by cool white fluorescent tubes. In vitro developed axillary shoots and single node of in vitro established mother explants were also used for mass multiplication of shoots.

In vitro rooting

In vitro produced shoots were transferred on the half strength MS medium containing 0, 1, 2, 5 and 10 μM of NOA (naphthoxy acetic acid) and 100 mg L^{-1} activated charcoal. Cultures were maintained at $28 \pm 2^\circ\text{C}$ under $35\text{--}40 \mu\text{mol m}^{-2} \text{s}^{-1}$ irradiance and 12 h d^{-1} photoperiod.

Results and discussion

Developmental stage and orientation of explants play an important role for establishment, induction and mass multiplication of shoots of *H. salicornicum*. Ruptured

Fig. 3 In vitro culture of mother explants of *H. salicornicum* on MS medium containing 10 μM BAP: **a** phase I, **b** phase II, **c** phase III. **d** In vitro rooting of shoots on half-strength MS medium containing 2 μM NOA and 100 mg L^{-1} activated charcoal

epidermis of mature shoots were observed to be major source of contamination and difficult to remove during surface sterilization of explants. Horizontally inoculated young green shoots were found to be more responsive than mature shoots on 10 μM BAP (Fig. 1b–f). The more efficient role of BAP on shoot proliferation has also been reported by Karolak et al. (2015). Approximately 82.51% horizontally inoculated young shoots responded on 10 μM BAP and produced average two axillary shoots of 0.7 ± 0.124 cm length per node (Fig. 2a, b). The advantage of horizontal positioning to improve propagation was also reported by Vieitez et al. (1992) in *Quercus rubra* L. and Liu et al. (2015) in *Jatropha curcas* L. Transportation and distribution of nutrients through weak node may be reduced and blocked in vertical position of explants in culture and that might led to growth inhibition and

necrosis. Further multiplication of shoots was achieved by sub culturing of in vitro regenerated axillary shoots and repeated sub culturing of mother explants on 10 μM BAP. Axillary shoot numbers were increased up to 17 shoots/node from mother explants at third subculture (Figs. 2c, d, 3a–c). The enhanced multiplication of shoots during subsequent sub culturing substantiates the earlier report on *Albizia chinensis* (Sinha et al. 2000) and *Haloxylon recurvum* (Dagla and Shekhawat 2005b). Horizontal position of shoots were found to be more responsive than vertical for induction of in vitro rooting. Intensive hairy roots were observed on horizontal shoots. About 30% of horizontally placed in vitro regenerated shoots were rooted on half strength semi-solid MS medium supplemented with 2 μM NOA + 100 mg L^{-1} activated charcoal after 3 weeks of inoculation (Fig. 3d). Half strength MS

medium was also used for induction of in vitro roots in *Salvadora persica* by Mathur et al. (2002). Present in vitro regeneration protocol of *H. salicornicum* can be a basis of mass plantation of this keystone species in the extreme arid regions.

Conclusion

Young green shoots in horizontal orientation were found to be more responsive than vertical orientation for activation of axillary shoot buds. Axillary shoot buds were activated on MS medium containing 10 μ M BAP. Adventitious roots with intense root hairs were induced on horizontally placed shoots on half strength MS medium containing 2 μ M NOA + 100 mg L⁻¹ activated charcoal.

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