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EFFECTIVE CALLUS INDUCTION AND PLANT REGENERATION IN THE CULTURES OF *HYDROCERA TRIFLORA* WIGHT & ARN.

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Abstract

In this study we report the development of effective in vitro systems for callus induction and mass propagation of *Hydrocera triflora*. Explants were collected from its natural habitat to derive plantlets through *in vitro* technique. Morphogenetic potential of somatic explants like shoot tip, axillary bud and leaf discs were studied by culturing them on Murashige and Skoog's medium, either alone or in combinations, with various plant growth regulators namely benzyl amino purine, indole-3-acetic acid, naphthalene acetic acid, kinetin and 2, 4-dichlorophenoxyaceticacid. Inoculated shoot tips and axillary bud explants expressed 100% shoot growth as well as multiple shoot formation with BAP+IAA (2.5mg/l each) treatment. Inoculated leaf disc explants shown significant callus initiation. Further caulogenesis and rhizogenesis were also noticed. The shoots rose from shoot tip and axillary bud explants were transferred to rooting medium containing the combinations of BAP+IAA, IAA+NAA and KIN+NAA to enhance root formation. Among, BAP+IAA and KIN+NAA combinations seems to be an effective for rhizogenesis with 100% rooting response. 2,4-D was reported as an effective hormone for callus initiation. The plantlets regenerated were acclimatized and propagated successfully.

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Key words: Callus, Caulogenesis, *Hydrocera triflora*, MS Medium and Rhizogenesis.

Introduction:

The micro propagation technique paves way for enormous production of plants at commercial scale as well as in a means of conservation. Now a days this technique practically plays a major role in the conservation of a wide range of rare (Holobiuc *et al.*, 2009), threatened (Pence, 2005), endemic (Nasircilar*et al*., 2011), endangered (Uzun *et al*., 2014) and recalcitrant species (Sarasan *et al.*, 2006). The protocols derived through micropropagation will be worthful and useful for mass multiplication of commercially important plant species in a faster pace (Panayotova *et al.*, 2008).

Hydrocera also known as 'the Marsh Henna', is a semi-aquatic plant, growing in still or stagnant water in pools, lakes, rice field, marshy places and ditches with lower part of the stem submerged. It is a perennial and can grow up to one meter in the tropics. The species has a fairly wide distribution in wet tropical and subtropical regions of the world. The plant is classified as 'Least Concern'in the IUCN Red List of Threatened Species. Economically, it is used in water gardening and organic water farming. The flowers are used to prepare a red dye for the fingernails (Grey-Wilson, 1980). Seeds produced by this species have shown a very low percentage of germination due to barriers like non-viability, impotency etc. Therefore, the propagation and conservation of this species is an impending need. So far, no effort has been made for *in vitro* propagation of this species. Hence, an attempt has been made for developing an efficient protocol for *in vitro* propagation of this species.

Materials and methods

Fresh disease free shoots and branches of *Hydrocera triflora* were identified from Thottiyodu pond, Nagercoil and were collected in polythene bags, wrapped with moist cotton to prevent desiccation. These shoots were brought to the laboratory which served as the source of explants. Healthy and young explants like shoot tips, axillary buds and leaf discs were collected. The excised explants were thoroughly washed in running tap water. Surface contaminants were removed by transferring the explants into water containing 1-3 drops of surfactant (Teepol) for 10 – 20 minutes followed by 70 percent ethyl alcohol for three seconds and then with 0.5 percent sodium hypochlorite solution for 3 – 5 minutes. The explants were again washed with double distilled sterile water for at least seven times. Finally these somatic explants were trimmed to 0.5 to 1 cm length and were inoculated aseptically on culture vials containing MS medium (Murashige *et* Skoog, 1962)with 3 percent sucrose and various PGR's. The pH of the medium was adjusted to 5.8 before adding 0.8 percent agar. The culture medium was autoclaved at 121°C for about 15 minutes. The inoculated culture vials were incubated at 24 ± 2°C under cool fluorescent white light (4000 lux, 16hr/d) and with 60 – 65 percent relative humidity. All experiments were repeated thrice.

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Shoot tips and axillary buds were cultured on MS medium fortified with cytokinin (BAP) and auxins (IAA, NAAand KIN) for direct shoot and callus induction. Shoot buds were regenerated directly from shoot tip and axillary bud explants which were transferred to the rooting medium fortified with same hormonal combinations for rhizogenesis. Leaf disc explants were cultured on the medium supplemented with 2,4-D and BAP for callus induction.All rooted plants were carefully washed with distilled water to remove the agar content. Further it was safely transferred to sterilized potting mixture and acclimatized under greenhouse conditions.

Results and Discussion

Response of shoot tip explant

Shoot tip explants cultured on MS medium supplemented with the hormonal combination of BAP+IAA (2.5mg/l each) induced prominent shoot proliferation with the maximum proliferation rate of 100%. The highest number of shoots (7.2 ± 2.94 shoots/explant) with the maximum shoot length of 2.74±1.36 cm was achieved (Fig. 1 & Fig. 5d).This result is similar to the previous finding by (Taha *et al*., 2009) in *Impatiens balsamina*. When the combination was altered, hormone dependent changes in bud proliferation were noticed. The combination when changed to IAA+NAA as well as KIN+NAA,cent percent proliferation rate was achieved but in a slower pace with reduced shoot length indicating the importance of BAP+IAA combination in shoot proliferation.

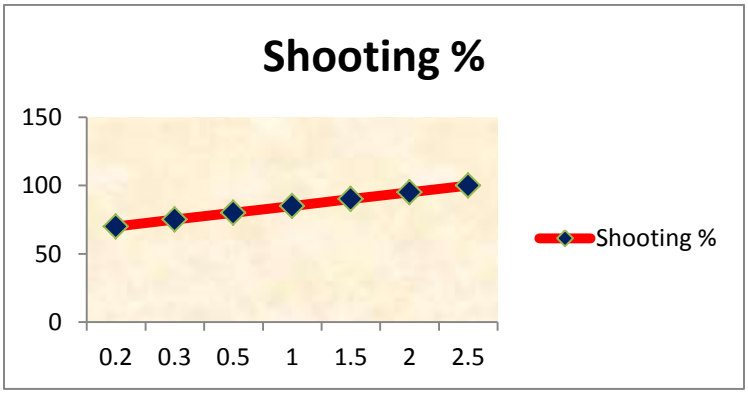

Figure 1. Effect of BAP+IAA on *in vitro* shoot proliferation from shoot tip explants of *H. triflora*. Data were recorded after 4 weeks of culture on MS medium.

Response of axillary bud explant

In axillary bud explants, the same hormonal combination of BAP+IAA (2.5mg/l each) showed profused shoot budding as well as multiple shoot formation (6.0±0.41 shoots/explant) in a faster pace at 100% response rate (Fig. 2 & Fig. 5e,f). The same response rate was also noticed with the

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combinations KIN+IAA (2mg/l each) and IAA+NAA (1mg/l each), while the shoot number was reduced to 1.6±0.89 and 1.87±0.25 respectively.The results obtained were in corroborative with the findings of (Herath *et* Wijesundara, 2010)in *Impatiens repens* and (Omaima *et al*., 2019) in *Impatiens balsamina*.

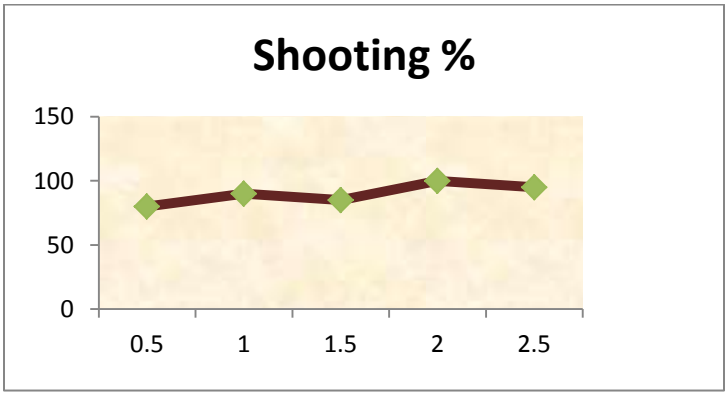

Figure 2. Effect of KIN+IAA on *in vitro*shoot proliferation from axillary bud explants of *H. triflora*. Data were recorded after 4 weeks of culture on MS medium.

Response of Leaf Disc explant

Leaf disc explants cultured on MS medium fortified with 2,4-D (1mg/l) shown callus initiation in a lower pace. When the concentration of the hormone was elevated, profuse callusing was reported with 2.5mg/l (Fig. 3 & Fig. 5a-c). Similar results were reported in *Impatiens balsamina* of Balsaminaceae where 2,4-D shown profuse callusing (Taha *et al*, 2009).

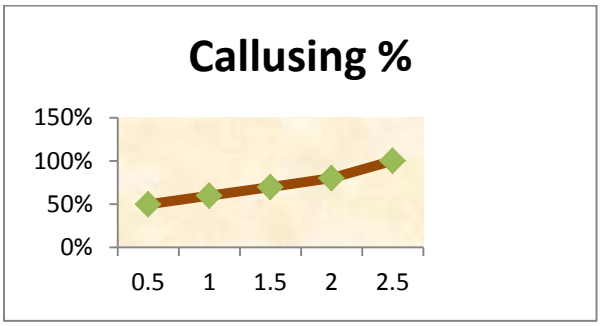

Figure 3. Effect of 2,4-D on callus induction from leaf disc explants of *H. triflora*. Data were recorded after 4 weeks of culture on MS medium.

***In vitro* rhizogenesis and acclimatization**

Successful rhizogenesis was achieved when the proliferated micro shoots from shoot tip and axillarybudexplants were supposed to be subjected to inoculation on rooting medium for

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prominent root production.Thus, for inducing rhizogenesis, the regenerated micro shoots from the shoot tip and axillarybud explants were transferred to MS medium supplemented with varied hormonal combinations viz., KIN+IAA, BAP+IAA and IAA+NAA. Maximum root number (25.6±4.81) was obtained by the addition of KIN+IAA (0.7mg/l each) with an average of 8.10±0.57cm root length compared with 5.33±0.81 roots in 2.5mg/l of BAP+IAA (16.6±7.56 cm) as well as 4.16±0.75 roots in 2.0mg/l IAA+NAA (14.0±5.96cm), (Fig. 4 & Fig. 5g,h). Altogether the results revealed that KIN+NAA (0.7mg/l each) and BAP+IAA (2.5mg/l each) in low concentrations induced rhizogenesis in *H. triflora*. These results were authenticated by the findings in *Impatiens platypetala* (Han, 1994), *Impatiens flanaganiae* (Josekutty*et al*., 1998). About 80 percent of the *in-vitro* regenerated plantlets were successfully transferred to soil in a green house (Fig. 5i).

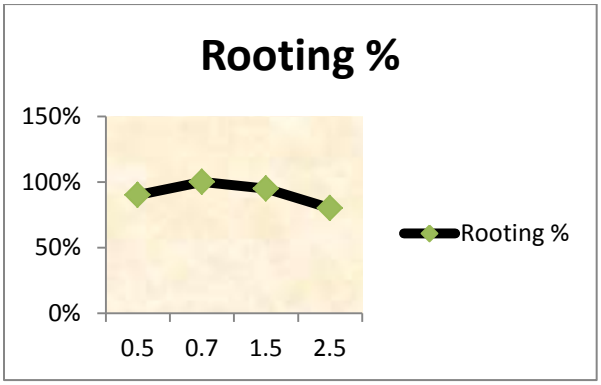

Figure 4. Effect of KIN+IAA on *in vitro* root induction from *in vitro* derived shoots of *H. triflora*. Data were recorded after 4 weeks of culture on MS medium.

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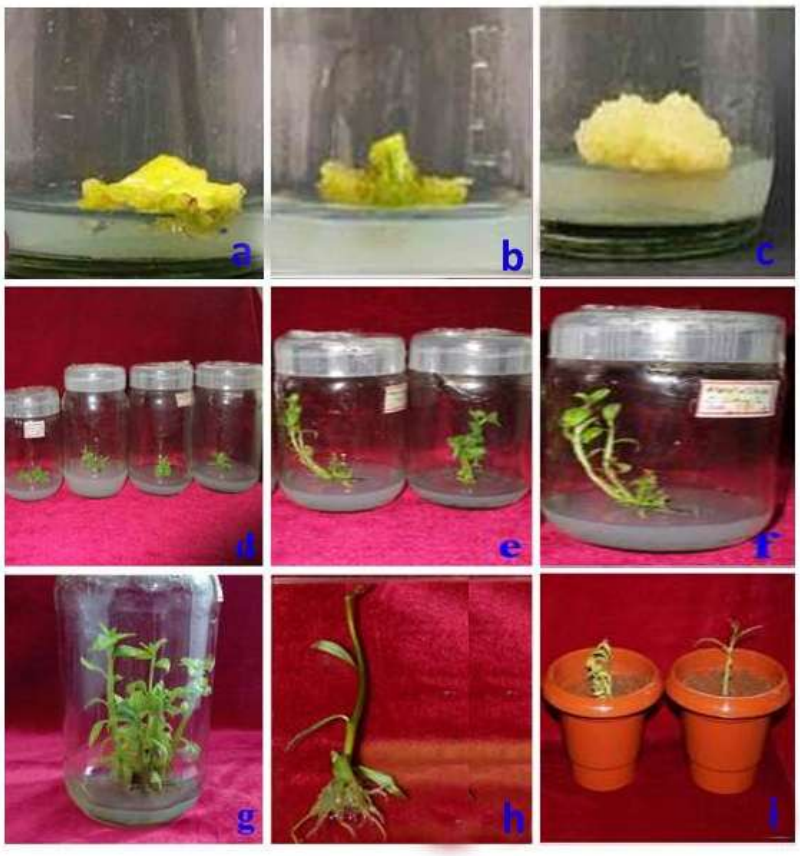

Figure 5. Micropropagation of *Hydrocera triflora* (**a-i**). **a-c:** Callus induction from leaf disc explants; **d**: Shoot proliferation from shoot tip explants; **e** & **f**: Shoot proliferation from axillary bud explants; **g**: Multiple shoot production and rhizogenesis. **h & i**: Acclimatization.

Conclusion

Hydrocera triflora having varied traditional values is now under severe threats due to over-exploitation, urbanization, habitat destruction and climatic change. Hence, conservation of this species is an impending need for the future. Considering this, the regeneration protocol derived here is much efficient and has the potential to propagate a good number of plantlets. Altogether, the study reveals that through direct morphogenesis, the shoot tip and axillary bud forms a potential explants which requires a hormonal combination of BAP and IAA for effective shoot production. The plantlets regenerated shown profuse rooting with the same hormonal combination.Thus the present investigation has resulted in the development of an eminent protocol for the mass multiplication of *Hydrocera triflora* within a short time duration, which could be employed for sustainable utilization as well as conservation of the species.

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