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PRELIMINARY STUDIES ON *IN VITRO* CULTURE OF *ATRIPLEX HORTENSIS* L.

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ABSTRACT

Atriplex hortensis L. commonly Known as Vust-e-Haak was regenerated from *in vitro* raised seedlings on MS ½ medium. Callus lines were induced from *in vitro* raised nodal segments and shoot tips explants on MS medium supplemented with BAP. Shoot tips and nodal segments when cultivated on combination NAA 7.5 µM+BAP 7.5 µM induced enhanced axillary branching. However, only elongation of main shoot and no axillary branching of shoot was observed on NAA (12.5µM) + BAP (12.5µM) from the nodal segment and shoot tips explants.

Keywords: *Atriplex hortensis*, shoot tips, nodal segments, callus, axillary branching

Abbreviations: MS- Murashige and Skoog; BAP-6–benzylaminopurine; NAA-α napthaleneacetic acid

INTRODUCTION

Atriplex species are members of the chenopods and include both C_3 and C_4 plants. The genus is quite variable and widely distributed. It includes many desert and seashore plants and halophytes, as well as plants of moist environment. There are more than 200 hundred species, most of which are highly tolerant to drought and salt. *Atriplex* species take up NaCl from saline soil and sequester it into the salt glands in their leaves. This is a useful characteristic and allows them to be used for revegetation in saline or arid /semi arid lands. *Atriplex* species contain high level of protein. Many species of the genus *Atriplex* are edible and are also excellent for livestock because of their favorable crude protein content (Mc Kell, 1994). However, the favored species for human consumption is *A.hortensis* (Garden Orache). The Garden Orache, also called Red Orache, Mountain Spinach, or French spinach, is an annual leaf vegetable with salty, spinach- like taste. *Atriplex hortensis* L. commonly called as vust-e- haak in Kashmir belongs to family Chenopodiaceae. It is widely grown in India and is much used for consumption in Kashmir.

Genetic transformation of *Atriplex* would be valuable for molecular farming and the production of the economically valuable proteins in saline and semi arid areas otherwise useless for agricultural crop production. It is in this direction that a preliminary attempt has been made to generate multiple shoots for successful micro propagation in *Atriplex hortensis* using nodal segments and shoot tips explants from *in vitro* raised seedlings plants.

MATERIAL AND METHODS

Seeds of *Atriplex hortensis* were washed with lab. detergent (Cedpol) containing a few drops of Tween-20 under running tap water for 5-10 minutes. These washed seeds after overnight soaking in distilled water, were surface

sterilized by 0.1% $HgCl_2$ for 15 minutes and finally rinsed 3-4 times with autoclaved double distilled water. Sterilized seeds were inoculated on MS ½ (1962) medium supplemented with 30 gm/l of sucrose. pH of the medium was adjusted to 5.8 and 0.8% agar was used as jelling agent. The cultures were maintained at 23 $\pm 2^0$C with 55-65% relative humidity and exposed to 16 hour photoperiod provided by cool fluorescent tubes (3000 lux). Shoot tips and nodal segments were excised from the seedlings, raised on the media, to initiate the primary cultures.

RESULTS

The seeds of *Atriplex hortensis* when inoculated on basal medium resulted in full fledged seedling formation (Fig.1a).

Callogenic response was observed in nodal and shoot tips excised from seedlings when cultured on MS ½ medium supplemented with different concentrations of BAP (Table 1). Pale yellow callus at the base along with the elongation of shoot was observed on MS+ BAP (1.0 µM). Green callus of very high degree was observed on BAP (7.77 µM) (Fig.1b). Pale yellow callus formation at the base of main shoot was observed at MS+ BAP (4.4µM) after six weeks of culture period (Fig.1c). All such calli were non-regenerative type.

More trials in this direction were conducted with two different BAP concentrations with NAA concentrations to see which concentration favours shoot multiplication

Response of *in vitro* raised nodal/shoot tip explants of *Atriplex hortensis* to various BAP and NAA concentrations is given in Table 2.

Table 1. Morphogenetic response of nodal segments and shoot tips of A. hortensis at various concentrations of BAP

INDUCTION MEDIUM	NATURE OF RESPONSE*	DEGREE OF CALLUS FORMATION
MS+BAP (1.0 µM)	Pale yellow Callus at the base	+
MS + BAP(1.11µM)	Pale yellow Callus at the base	+
MA+BAP (4.44µM)	Pale yellow Callus at the base	++
MS+BAP (7.77µM)	Green Callus at the base	+++

Data scored at the end of six weeks of culture period, * Means of 10 replicates

+ low growth, ++ Moderate, +++ high

Table 2: Effect of BAP and NAA on the nodal segments and shoot tips explants of *A. hortensis*

GROWTH MEDIUM	RESPONSE	DEGREE OF CALLUS FORMATION
MS+BAP (7.5μM) + NAA(7.5μM)	Axillary branching and elongation of main shoot	+
MS + BAP (12.5μM) +NAA (12.5μM)	Elongation of main shoot and no axillary branching	+

+low growth

On MS+BAP (7.5μM) + NAA (7.5μM) elongation of shoots with enhanced axillary branching was observed along with low callus formation at the basal end (Fig. 1d). However elongation of shoots without axillary branching was registered on MS+BAP (12.5 μM) + NAA (12.5 μM).

DISCUSSION

Present preliminary findings reveal information regarding morphogenetic potential of nodal segments and shoot tips explants of *Atriplex hortensis*. Enhanced axillary branching was observed by cultivating shoot tips and nodal segments on medium supplemented with combination of 7.5 μM BAP and 7.5 μM NAA. In contrast Uchida *et al.* (2003) have reported that *A gmelini* plants were regenerated via organogenesis from hypocotyl explants. Shoots were regenerated from the callus lines on L.S. medium supplemented with 20 μM TDZ and 0.1μM α -napthalenacetic acid under high – intensity light. However TDZ, which is having cytokinin like activity was, reported to induce not only adventitious and/ or axillary shoot production through organogenesis, but also somatic embryogenesis in apple, wheat, barley and mulberry (Saito and Suzuki 1999; Sugimura *et al.*, 1999; Shan *et al.*, 2000) which is not in line with present studies. The combination of TDZ and NAA at various concentrations was tested to promote regeneration in *Atriplex gmelini* (Uchida *et al.*, 2003). These studies run parallel to our findings as TDZ has cytokinin like activity and with NAA proves effective. Different degrees of callus formation was observed on various concentrations of BAP. However Uchida *et al.*, reported that frequency of callus formation was highest at 1μM BAP and 5μM NAA. Callus proliferation being most prominent on MS medium supplemented with 9.3 μ*M* of 6-furfurylaminopurine (kinetin) and 3.39 μ*M* 2,4-dichlorophenoxyacetic acid (2,4-D) was reported by Al-Khayri *et al.* (1991) in spinach. In present study callus formation was highest with BAP (7.7 μM).

Present findings indicate that shoot tips and nodal explants of *Atriplex hortensis* possess the potentiality to produce enhanced axillary

branching which can be used for plantlet formation after following proper rooting procedure in isolated shoots. Micropropagation of *Atriplex* species is very important for the genetic transformation to elucidate the mechanism of salinity tolerance in halophytes.

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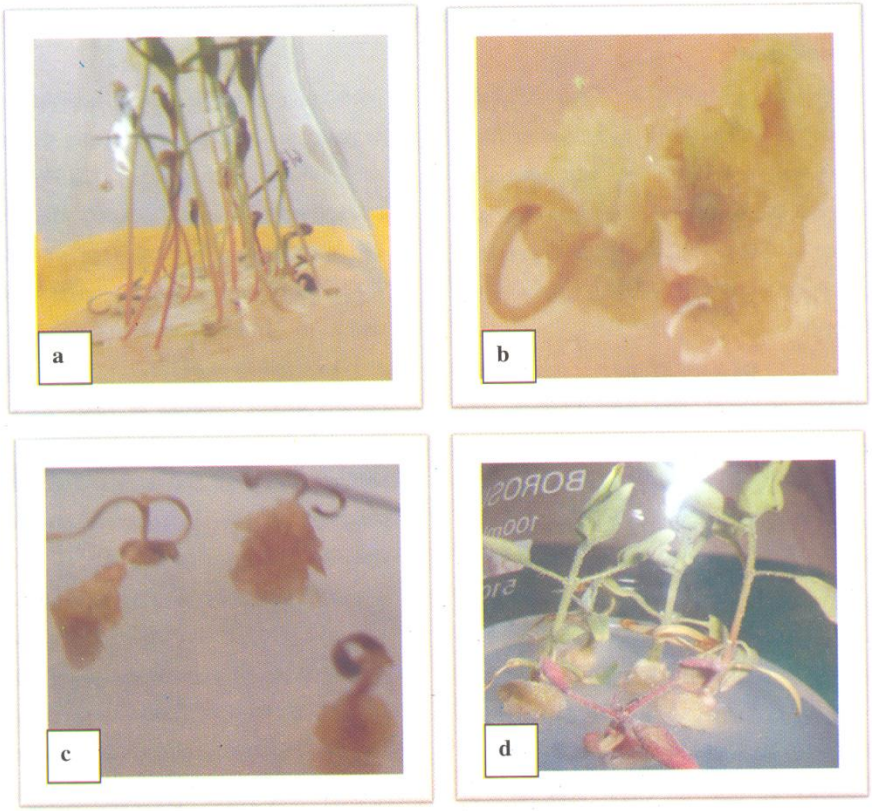

Fig.1 (a-d) ***In vitro*** **culture of** ***Atriplex hortensis*** **L.**

(a) ***In vitro*** **seed germination and seedlings formation on MS(1/2) basal medium (after 2 weeks)**
(b) Green callus formation in nodal/shoot tip explants on MS+ BAP (7.77 µM) (after six weeks)
(c) Callus formation in nodal/shoot tip explants on MS+ BAP (4.4 µM) (after six weeks)
(d) Axillary branching and elongation of shoots on MS+ BAP (7.5µM)+ NAA (7.5µM) (after 10 weeks.)

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