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In Vitro Conservation of Medicinally Important Climbing Shrub *Maerua Arenaria* Hook. F. and Thomson

K. Thirupathi[1], Md. Ghani[2], V. Suresh[3], T. N. Swamy[4], G. Rajender[5] and Md. Mustafa[5*]

Plant Tissue Culture and Molecular Taxonomy Laboratory, Dept. of Botany, Kakatiya University, Warangal, India

**Corresponding Author: mustafarz67@gmail.com, Tel: +91-9440582638*

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Abstract- Caper family belongs to the order Brassicales comprises various important medicinal plants distributed in tropical countries. *Maerua* is the second largest genera in this family. *Maerua arenaria* is a wild climbing shrub having high medicinal value, due to over exploitation it becomes threatened. *In vitro* propagation technique has been adopted for conserving the valuable medicinal plant by using plant growth regulators. Initially the nodal explants were inoculated on MS medium supplemented with 4.0 mg/l BAP and 2.0 mg/l NAA for axillary bud proliferation. Multiple shoots (5-6 shoots per explant) were recorded after 34 days on MS medium with 2.0 mg/l BAP and 2.0 mg/l IAA. Since the shoots are small, they were transferred on MS medium containing 2.0 mg/l BAP and 2.0 mg/l GA_3 for shoot elongation. These well developed elongated shoots were transferred to MS medium supplemented with 2.0 mg/l IBA for rooting. These *in vitro* developed plantlets were acclimatized in green house and successfully transplanted to natural condition and 78% of plants were survived.

Keywords: Conservation; *In vitro* Propagation; MS medium; Plant growth regulators; Shoot elongation.

I. INTRODUCTION

India is richly constituted with a wide variety of plants having high medicinal values. These plants are widely used whether directly as folk medicines or indirectly as pharmaceutical application of modern medicine (Pandey *et al*., 2013). The capparidaceae is commonly known as the caper family belongs to the order Brassicales comprises various important medicinal plants distributed in tropical and subtropical India, Pakistan, Africa and Saudi Arabia. It consists of 33 genera and about 700 species. The largest genera are *Capparis* (about 150 species), *Maerua* (about 100 species), *Boscia* (about 37 species) and *Cadaba* (30 species).

Maerua arenaria is a medicinally important climbing shrub commonly called morinika in telugu belongs to the family capparaceae. It is growing up to 3m. long, commonly found growing wild in scrubland. Leaves are oblong, ovate, entire. Flowers usually corymbose racemes, greenish white, pedicellate, look beautiful with mainly the greenish stamens radiately out. Sepals 4, ovate-elliptic, acute (Fig.1A). Fruit cylindrical torulose or irregularly many knotted, pale brown, often somewhat twisted (Fig.1B) and each knot is one-seeded (Fig.1C).

Maerua arenaria is a rich source of phytochemical ingredients such as phytosterols, alkaloids, saponins, glycosides, carbohydrates and aminoacids. Since time immemorial plants have been used for curing various diseases in human being and animals (Usha *et al*., 2016). Ethnomedical survey reveals that it is used to cure various diseases such as fever, stomachache, skin infection, urinary calculi, epilepsy, pruritis, rigidity in lower limbs and abdominal colic (Moglad *et al*., 2014). Leaves and roots are used to diabetes, stimulant, alternative (Savitramma *et al*., 2011) and root tubers are traditionally given in sterility, thathu viruthi (increase sperm) and aphrodisiac action (akhila *et al*., 2014). *Maerua arenaria* is over harvested for its commercial use, accompanying with the destructive harvest of underground parts from wild for its aphrodisiac property, which is ensuing results in the loss of their existing populations. To prevent the extinction of this medicinal plant, there is an urgent need for conservation of this plant, due to this reason we have adopted *in vitro* propagation (micropropagation) method for the conservation.

II. MATERIALS AND METHODS

Plant material, explant preparation and surface sterilization:

Maerua arenaria plant materials were collected from the Mulugu forest area of Jayashankar Bhupalpally district, Telangana State, and planted in the field area of Botanical garden, Department of Botany, Kakatiya University, Warangal. After potting, the arose shoots were used as a source of explants for this experiment. These shoots were surface sterilized to remove the surface borne microorganisms, explants were thoroughly washed under running tap water for 10 minutes accompanied with 2-3 drops of tween 20 and then surface sterilized with 0.1% mercuric chloride for 3-4 minutes after that these explants were washed with double sterile distilled water to remove $Hgcl_2$ completely. These shoots were then placed on a sterilized filter paper to remove moisture and then aseptically cut into approximately 1cm nodal explant and inoculated on MS medium. All the surface sterilization steps were carried out under laminar air flow chamber.

Media preparation, *in vitro* shoots induction and maintenance of culture conditions:

For all experiments, Murashige and Skoog medium (1962) was used with 3% (*w/v*) sucrose as carbon source then the P^H was adjusted to 5.6±0.2 before adding 0.8% (*w/v*) of agar. The medium was then autoclaved at 121^0 C for 15-20 minutes. All growth regulators were added before autoclaving for axillary bud proliferation and shoot induction. Surface sterilized nodal explants of *Maerua arenaria* were inoculated aseptically on MS medium added with different concentrations of phytohormones like BAP (0.5-5.0 mg/l) in combination with 2.0 mg/l NAA and 2.0 mg/l BAP in combination with IAA (0.5-3.0 mg/l). Cultures were incubated at 25 ± 2^0C in 16/8 hrs photoperiod provided by cool and white fluorescent tubes with 55±5% RH.

Shoot multiplication and elongation:

The *in vitro* raised shoots were individually subcultured on fresh MS medium fortified with 2.0 mg/l BAP in combination with IAA (0.5-3.0 mg/l) and 2.0 mg/l BAP in combination with GA_3 (0.5-3.0 mg/l), 3% (*w/v*) sucrose and 0.8% (*w/v*) agar.

***In vitro* root induction and acclimatization of cloned plantlets:**

The *in vitro* raised shootlets (about 5-6 cm in length) were excised and transferred on MS medium fortified with different concentrations of IBA (0.2-3.0 mg/l) for rooting. After three weeks plantlets were rooted on MS medium supplemented with 2.0 mg/l IBA. *In vitro* raised plantlets were hardened in polycups containing a mixture of sand, vermicompost and black soil. These plants were acclimatized in a culture room at 25 ± 2^0C in 16/8 hrs photoperiod provided by cool and white fluorescent tubes and 55±5% RH for two weeks. These plantlets were then kept in green house at 80-90% RH, 28 ± 2^0C before subsequent transfer to field.

III. RESULTS AND DISCUSSION

Among the methods for plant micropropagation, the axillary bud proliferation is the most suitable to guarantee genetic stability of the regenerated plants obtained. Generally axillary buds are cultured to regenerate multiple shoots without intervention of callus phase. We optimized axillary bud proliferation, multiple shoot induction, shoot elongation and *in vitro* rooting techniques for mass *in vitro* clonal propagation without interference of callus in *Maerua*. This type of experiments were already conducted in *Fragaria indica* (Bhatt & Dhar., 2000), *Acacia mearnsii* (Marguerite *et al*., 2001), *Santalam album* (Sanjaya *et al*., 2006) and *Crataeva religiosa* (Basu *et al*., 2009). Initially nodal explants were inoculated on MS medium supplemented with different concentrations of BAP (0.5-5.0 mg/l) with 2.0 mg/l NAA (Table – 1). Higher percentage of response (80%) was achieved on MS medium supplemented 4.0 mg/l BAP in combination with 2.0 mg/l NAA after one subculture from the nodal explants induced axillary buds after four weeks with each culture and subculture period (Fig.1D). Similar results were obtained in *Dalbergia latifolia* (Boga *et al*., 2012), *Echinocereus cinerascens* (Elias *et al*., 2015). However, lower and higher concentration of BAP with 2.0 mg/l NAA has decreased the percentage of response. MS medium fortified with 3.0 mg/l BAP and 2.0 mg/l NAA has shown moderate response (66%). When these axillary buds were cut from the basal end and sub cultured on MS medium fortified with different concentrations of IAA (0.5-3.0 mg/l) and 2.0 mg/l BAP. With an average of 5-6 number of shoots were achieved on MS medium with 2.0 mg/l BAP and 2.0 mg/l IAA with maximum percentage of response (78%) after 24 days of culture (Fig.1E). The regeneration of shoots from nodal explants has also been encountered in *Withania somnifera* (Kumar *et al*., 2011), *Stevia rebaudiana* (Thiyagarajan & Venkatachalam., 2012), *Toddalia asiatica* (Anand *et al.*, 2015). However, lower and higher concentration of IAA in combination with 2.0 mg/l BAP has decreased percentage of response, MS medium supplemented with 2.0 mg/l BAP and 1.5 mg/l IAA showed moderate response (56%). The obtained small shoots were transferred on MS medium supplemented with various concentrations of GA_3 (0.5-3.0 mg/l) and 2.0 mg/l BAP. Maximum (84%) percentage of shoots were elongated on MS medium with 2.0 mg/l BAP and 2.0 mg/l GA_3 (Fig.1F). Mostly GA_3 alone or in combination with BAP or IAA is suitable for shoot elongation, similar results were obtained in *Camellia sinensis* (Gonbad *et al*., 2014), *Eclipta alba* (Dhaka & Kothari., 2005). However, lower and higher concentration of GA_3 together with 2.0 mg/l BAP has decreased percentage of response for shoot elongation, MS medium augmented with 2.0 mg/l BAP combined with 1.5 mg/l GA_3 obtained moderate response (66%). The well

developed *in vitro* shoots were transferred on MS medium supplemented with different concentrations of IBA (0.5-3.0 mg/l) for rooting. The maximum (70%) percentage of roots were observed on MS medium supplemented with 2.0 mg/l IBA after 18 days of inoculation (Fig.1G). Similarly IBA treatment was given for root induction in *Warburgia ugandensis* (Akwatulira *et al*., 2011), *Eclipta alba* (Dhaka & Kothari., 2005), *Sapindus mukorossi* (Philomina & Rao., 2000), *Bambusa glaucescens* (Shirin & Rana., 2007), *Andrographis paniculata* (Purkayastha *et al*., 2008). However, lower and higher concentration of IBA has decreased percentage of response for rooting, MS medium supplemented with 1.5 mg/l IBA showed moderate response (65%) of root induction. The *in vitro* rooted plantlets were washed with sterile distilled water and transferred to small plastic pots containing sand, vermicompost and black soil covered with polythene bags (Fig.1H). Then the plantlets were maintained under greenhouse for one week and then transferred to land with 78% survival (Fig.1I). This is the short method of conservation of Maerua arenaria have been achieved over the previous methods in other species.

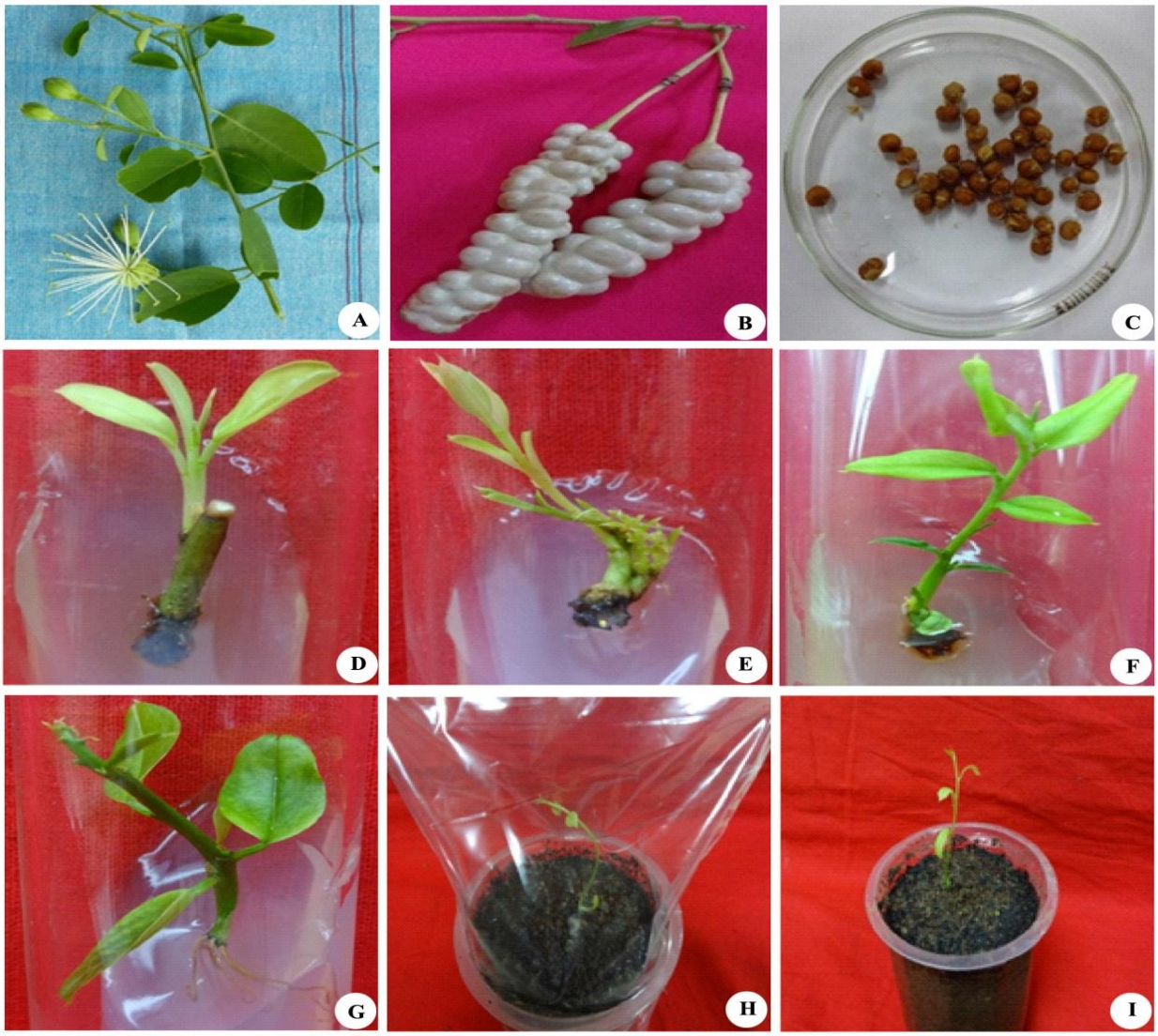

Figure 1. Morphology and Tissue culture studies in *Maerua arenaria*

A - Small twig with flower; **B** - Fruits; **C** - Seeds; **D** - Axillary bud proliferation from nodal explant on MS medium supplemented with 4.0 mg/l BAP and 2.0 mg/l NAA; **E** - Induction of multiple shoots from nodal explant on MS medium fortified with 2.0 mg/l BAP and 2.0 mg/l IAA; **F** - Shoot elongation on MS medium augmented with 2.0 mg/l BAP and 2.0 mg/l GA3; **G** - Induction of oots on MS medium supplented with 2.0 mg/l IBA; **H** - Hardening plants covered with polythene bag; **I** - Hardened plant in green house

Table-1: Effect of plant growth regulators on nodal explants of *Maerua arenaria*

Plant growth regulator mg/lit					Morphogenic response	Percentage of explant response (%)
BAP	NAA	IAA	GA_3	IBA		
0.5	2.0	--	--	--	NR	NR
1.0	2.0	--	--	--	NR	NR
1.5	2.0	--	--	--	ABP	25
2.0	2.0	--	--	--	ABP	50
2.5	2.0	--	--	--	ABP	58
3.0	2.0	--	--	--	ABP	66
3.5	2.0	--	--	--	ABP	75
4.0	**2.0**	--	--	--	**ABP**	**80**
4.5	2.0	--	--	--	ABP	32
5.0	2.0	--	--	--	ABP	24
2.0		0.5	--	--	MS	16
2.0		1.0	--	--	MS	42
2.0		1.5	--	--	MS	56
2.0		**2.0**	--	--	**MS**	**78**
2.0		2.5	--	--	MS	34
2.0		3.0	--	--	MS	25
2.0		--	0.5	--	SE	NR
2.0		--	1.0	--	SE	32
2.0		--	1.5	--	SE	66
2.0		**--**	**2.0**	--	**SE**	**84**
2.0		--	2.5	--	SE	25
2.0		--	3.0	--	SE	16
--		--	--	0.5	R	NR
--		--	--	1.0	R	34
--		--	--	1.5	R	65
--		--	--	**2.0**	**R**	**70**
--		--	--	2.5	R	48
--		--	--	3.0	R	22

*Where NR- No Response, ABP – Axillary bud proliferation, MS – Multiple shoot induction, SE – Shoot elongation, R – Rooting.

IV. CONCLUSION

The present study provides an efficient plant regeneration method for *Maerua arenaria*, through nodal explants with good survivability.

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