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

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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 GA₃ 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 arisen 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 Hgcl₂ 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 pH was adjusted to 5.6±0.2 before adding 0.8% (w/v) of agar. The medium was then autoclaved at 121°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 IBA in combination with IAA (0.5-3.0 mg/l). Cultures were incubated at 25±2°C 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 GA₃ (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°C 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°C 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 (Elías 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 IAA 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 GA₃ (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 GA₃ (Fig.1F). Mostly GA₃ 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 GA₃ 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 GA₃ 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.
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 | GA₃ | 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 | 58 |
| **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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