Research Article

# *In-Vitro* Propagation of *Neurada procumbensl* L (Chipri Booti): An Endangered Medicinal Plant from Cholistan Desert

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**Abstract** | Present investigations were carried out with a view to optimize an in-vitro culture protocol for propagation of an endangered medicinal plant *Neurada procumbens* L. from Cholistan desert in Pakistan. Nodal segments from healthy grown plants were used as explants and cultured on standard Murashige and Skoog (MS) medium supplemented with different concentrations and combinations of benzyl amino purine (BAP) or kinetin for shoot induction and Indole acetic acid (IAA) for primary shoot and Indole Butyric acid (IBA) for root proliferation. Best shoot proliferation (4.48 per explant) was observed in MS medium containing 0.2/0.4 mg/L BAP/IAA combination. For rooting of the micro shoots, MS medium was supplemented with 1.0 mg/l IBA exhibited best results with 4.69 mean value of roots per shoot at an average root length of 3.86 cm. After acclimatization and transplantation, 90% of the In- vitro derived plants were found healthy in ex vivo conditions.

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## Introduction

The Cholistan desert of Pakistan is considered as an important region for its endemic and opulent in seasonal and perennial medicinal plants due to punitive environment. The desert vegetation with medicinal potential has extremely been exploited for herbal therapeutic preparations (Chaudhary et al., 2004). Many plants in Cholistan desert especially *Neurada procumbens*, *Aerva javanica*, *capparis deciduas*, *alhagi maurorum*, *calotropis procera*, *Cleome brachycarpa*, *Dipterygium glaucum*, *Ziziphus mauritiana,* and *Boerhavia procumbens* are being used as medicinal plants for human (Immanuel and Elizabeth, 2009; Jabeen et al., 2009; Khan, 2009; Marashdah and Al-Hazimi, 2010; Qureshi et al., 2010; Hameed et al., 2011) and livestock (Khan et al., 2009) ailments by local inhabitants (Qureshi et al., 2005). A number of medicinal plant species have been declared endangered due to grazing, over collection and low propagation rate under intensive environmental stresses (Hameed et al., 2011). The Neurada procumbens L. (Neuradaceae) ordinarily acknowledged as "Chipri Booti" is also an important curative herb of the Cholistan region. This is a ruderal, annual herb. Its distribution range spans from North Africa and the Mediterranean region

across the Middle East to Afghanistan, Pakistan and north-western India (Marwat and Siddiqui, 2013). Star shaped dried fruit in powdered form is used with rose water in summer as cooling agent and along with dried nuts in winter as nerve tonic (Qureshi et al., 2010). Kapoor and Kumar (2013) reported that the use of *N. procumbens* in Barmer district of Rajasthan as cure for heat stroke in summer. It is also a common plant of Arabian Peninsula used for therapeutic purposes. The climatic change (drought), over grazing, indiscriminate collection, poor seed setting and germination of the flora of Cholistan region has main causes of threat for many valuable medicinal plants including the *N. procumbens*. Exploitation of modern biotechnological approach to conserve the endangered halophytic medicinal herbs, aromatics plants and forage grasses as strategy to sustain plant biodiversity is the need of hour (Dharmandra et al., 2010). The *in vitro* conservation technique of a large number of threatened and endangered plants proved very effective (Jhonson et al., 1997; Jaffar et al., 2011). The *in vitro* study was carried out to optimize micro propagation for *N. procumbens*, with the aim to conserve the flora of Cholistan desert (Southern Province of Punjab, Pakistan).

## Materials and Methods

The present study was conducted in plant tissue culture lab National Agricultural Research Centre (NARC), Islamabad and material was collected from Cholistan Institute of Desert Studies (CIDS), Islamia University Bahawalpur in June 2015.

### *Collection of explants and preparation of Medium*

Tender twigs of *Neurada procumbens* L. were collected from actively growing shoots, raised under controlled conditions at Cholistan Institute of Desert Studies (CIDS), Islamia University, Bahawalpur, Pakistan. Nodal explants defoliated and sectioned into 2-3 nodal segments and washed with tap water to remove the dust and soil particles and rinsed with distilled water. Later the cuttings were surface sterilized with 70% commercial bleach (Clorox 5.75% NaOCl) to which few drops of- Tween-20 were added and shake it for 5 minutes. Traces of bleach were removed by four times washing with autoclaved distilled water under aseptic conditions in laminar flow chamber and kept on sterile filter paper (Wattman 1) in petri dishes for drying. The basal medium used for this experiment was MS (Murashige and Skoog, 1962) medium containing 20% sucrose and supplemented with different concentrations and combinations of BAP and IAA for shoot proliferation, regeneration and rooting. The pH of media was adjusted at 5.7± 0.2 and autoclaved it after adding 1.8g/L gellan gum powder to solidify the medium.

### *Initiation of explants in culture medium for shoot proliferation*

The nodes of explant were cut into small pieces of 0.5-1 cm with the help of sterilized surgical blade and cultured on basal medium supplemented with different combinations of BAP (0.2mg/L, 0.4mg/L, 0.6mg/L) and IAA (0.2 mg/L, 0.4 mg/L, 0.6 mg/L ) poured in glass tubes. Cultures were incubated at 25 ± 2˚C with a photoperiod of 16 h at 1500 - 2000 lux of cool white fluorescent light inside growth chamber for shoot induction. Color, size and texture of shoots were monitored on regular intervals. All the cultures were transferred to fresh medium after 2-3 week duration. Morphological changes were recorded on the basis of visual observations at 3-week intervals.

### *Rooting of mature shoots and hardening*

Shoots of 4-5 cm in height with two or three leaflets derived from cultures were separately transferred to MS medium containing different concentrations of IBA (0.0 mg/L, 0.5 mg/L, 1.0 mg/L, and 1.5 mg/L) and IAA (0. mg/L 0, 0.5 mg/L, 1.0 mg/L, and 1.5 mg/L) separately for rooting. The observation was taken at regular intervals of one week up to 6 weeks of culture and the obtained root number and their length were recorded.

### *Acclimatization*

After the formation of complete rooted plantlet, they were subjected to ex vitro hardening. The rooted plantlets were washed with tap water to remove agar and shifted to pots having sterile soil and sand mixture (1:2). Potted plants were transferred to greenhouse for 25-30 days under partial shade and irrigation was done with three days interval. Data was recorded for number of stem nodes, number of leaves, leaf length, number of roots, root length.

## Results and Discussion

Growth hormones *i.e.*, Cytokinins and Auxins plays an important role in proliferation of shoots and growth under invitro condition and they are found in all higher plants with maximum concentration at mer-

istematic region and areas of continuous growth such as roots and developing leaves (Dharmendra et al., 2010). These hormones promotes cell division, stimulates shoot initiation/bud formation and leaf expansion when use in tissue culture (Meena et al., 2014).

In present study cytokinins and auxins were used as BAP and IAA respectively with different concentrations and combinations in MS media to induce shoot proliferation and auxiliary bud initiation in (*Neurada procumbens* L.). Combination of BAP and IAA i.e., 0.2 mg/L BAP and 0.4 mg/L IAA was found to be most effective in producing maximum number of stem nodes (Table 1). Dharmendra et al. (2010) and Yadav et al. (2011) also narrated this combination of growth hormones most effective for shoot proliferation.

**Table 1:** *Mean Values for the Effect of BAP/IAA.*

| Media | BAP/IAA Concentrations mg/L | Number of shoots | Number of Leaves | Shoot length (cm) |
|---|---|---|---|---|
| 1 | 0.2/0.2 | $3.16^{c-e}$ | $5.27^{b-d}$ | $6.34^{de}$ |
| 2 | 0.2/0.4 | $4.48^{a}$ | $6.29^{a}$ | $7.46^{a}$ |
| 3 | 0.2/0.6 | $3.87^{b}$ | $5.89^{ab}$ | $7.33^{ab}$ |
| 4 | 0.4/0.2 | $3.88^{b}$ | $5.433^{bc}$ | $7.22^{ab}$ |
| 5 | 0.4/0.4 | $3.59^{b}$ | $5.23^{cd}$ | $7.20^{ab}$ |
| 6 | 0.4/0.6 | $3.59^{b}$ | $5.07^{cd}$ | $6.99^{a-c}$ |
| 7 | 0.6/0.2 | $3.56^{bc}$ | $4.99^{cd}$ | $6.82^{b-d}$ |
| 8 | 0.6/0.4 | $3.51^{b-d}$ | $4.81^{cd}$ | $6.56^{c-e}$ |
| 9 | 0.6/0.6 | $3.12^{de}$ | $4.88^{cd}$ | $6.28^{de}$ |
| 10 | Control | $3.03^{e}$ | $4.64^{d}$ | $6.06^{e}$ |

**Means with same letters are not statistically different.*

### *Number of shoots*

The number of shoots per explant was found higher in nodal segments compared to shoot tip of explants (*Neurada procumbens* L.). Shoot tips cultured in MS media supplemented with different combinations of BAP and IAA showed a highly significant difference for shoot induction. Table 1 showed that out of various concentrations of BAP and IAA tested, MS medium containing 0.2mg BAP with 0.4mg and 0.6mg IAA are the most optimum concentrations that showed maximum number of shoot induction with highest mean value 4.48 and 3.87 respectively. Lorente and Apostolo (2013) also found this combination most effective during micro-propagation of jojoba. An indirect relationship was observed as the concentration of BAP increase because the mean value for the number of shoots decrease from 3.88 to 3.08 shoots per plant. Khan et al. (2009) also observed the same results in brassica family.

### *Number of leaves*

Number of leaves is an important growth parameter to quantify the effect of shooting hormones for the invitro propagation. In *Neurada Procumbens* highly significant differences was documented for number of leaves against growth medium with different concentrations of hormone BAP and IAA. Highest mean value for number of leaves (6.29cm) was observed in 0.2mg/0.4mg per liter concentration of BAP/IAA respectively followed by the combination with 0.2mg/0.6mg per liter concentrations of BAP/IAA (5.89cm). Whereas the shooting meia with BAP/IAA combination 0.6mg/0.4mg per liter concentration was at the bottom for number of leaves during micro-propagation. Rahman *et al.*, (2004) found similar results in the experiment of BAP for invitro regeneration of Banana (*Musa*) for maximum number of leaves.

### *Shoot length*

Highly significant differences were recorded for shoot length and hormone concentration. In Table.1 mean values for the effect of cytokinin (BAP) concentrations showed that shoot length increased directly as BAP concentration increased. Whereas with the gradual increase in IAA, the shoot length decrease that showing the inversly proportional effect. Rahaman et al. (2004) observed similar results. They observed longest shoot in the treatment 5.0 mg/l BAP (3.62 cm) followed by 4.0 mg/l BAP (3.40 cm) using in BARI Banana. Smitha et al. (2005) reported that when 0.05 mg l-1 kinetin was added in MS mediun which already had 1.0 mg/l of BAP, the production of dark green healthy shoot enhanced. The best combination of BAP and IAA is 0.2mg/0.4mg per liter for maximum shoot length i.e. 7.46cm noted. Whereas when BAP and IAA used in high concentration i.e. 0.6 mg/0.6mg showed lowest mean value for shoot length. It was also found that explant showed better shoot formation response in liquid medium as compared to the solid medium. In liquid medium, the close contact of the tissue with the medium may facilitate the uptake of nutrients and phytohormones, leading to better shoot growth (Sandal et al., 2001).

### *Effect of rooting hormone*

**Number of Roots**: IBA (indole butyric acid) was the main rooting hormone during the protocol formation

of micro propagation of *Neurada procumbens* plants while IAA was also used. Qurainy et al. (2013) narrated IBA as best rooting hormone in *Ochrademus baccatus*. Afolayan et al. (2014) also supported the opinion that by adding optimum concentration of IBA in culture media, maximum number of roots observed during invitro propagation of medicinal plants. In the regeneration of *Neurada Procumbens* mean values for number of roots and root length with different concentrations of rooting hormones i.e. IAA and IBA showed a significant difference (Table 2). Maximum number of roots was found with 1.0mg/0.0mg IBA/IAA concentrations. Tadhani et al. (2003) reported initiation of rooting within 6-7 days and obtained maximum number of roots on medium supplemented with 1.0 mg/L of IBA. Ferreira and Handro (1988) also reported that addition of auxin to the rooting medium (especially 0.1 mg/L IBA) favored root formation. Smitha et al. (2005) also recorded almost same results when used modified MS medium supplemented with 1.5 mg /l indole-3-butyric acid. Whereas the mean values for number of roots decrease with increase the concentration of IBA in the rooting media of *Neurada Procumbens*. Lowest number of roots was observed when IAA used at 1.0mg/L in rooting medium (3.59 cm) but the mean again increase with increase in IAA concentration 1.5mg/L recorded (3.93cm). More or less same results were also observed by Venis et al. (1996). It indicated that changes may be due to different species.

**Table 2:** *Mean Values for the Effect of Rooting Hormone (IBA/IAA) on Number of Roots and Root length.*

| IBA/IAA (mg/L) | Number of Roots | Root Length (cm) |
|---|---|---|
| 0.5/0.0 | 3.66[c] | 3.05c |
| 1.0/0.0 | 4.69[a] | 3.86a |
| 1.5/0.0 | 4.26[b] | 3.75a |
| 0.0/0.0 | 4.01[b] | 3.56ab |
| 0.0/0.5 | 3.73[c] | 3.19bc |
| 0.0/1.0 | 3.59[cd] | 3.04c |
| 0.0/1.5 | 3.93[d] | 2.84c |

**Means with same letters are not statistically different.*

**Root length:** Root length developed by the plantlets was influenced considerably by different concentration of rooting hormones IAA/IBA during micro-propagation (Meena et al., 2014) and showed siginificant significant differences in mean values for root length *N. Procumbens* (Table 2). Rooting media with 1.0mg IBA proved best concentration with maximum mean value of root length (3.86cm) followed by rooting media with 1.5mg IBA (3.75cm). Molla et al. (2004) reported that 1.5 mg/l IBA concentration is more suitable for invitro rooting and root length (Figure 1). Habiba, (2002), Khanam et al. (1996) and Ali, (1996) also reported more or less similar results. Hence present results partially agreed that IBA induce positive results on the root length when use in rooting media.

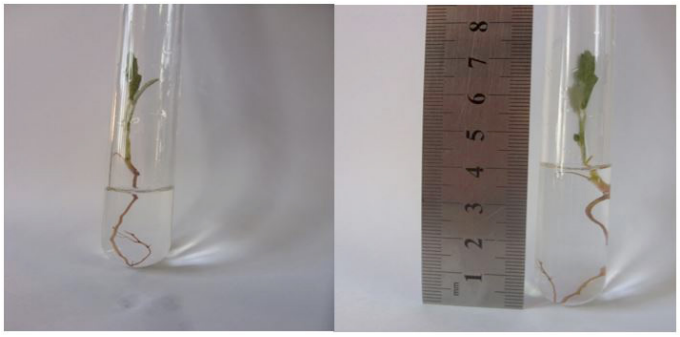

**Figure 1:** *Intitro rootiung.*

**Hardening and acclimatization**: Well developed In vitro plants were shifted in different peat moss for hardening and acclimatization of micro propagated plants of (*Neurada procumbens* L.) in the screen house was achieved with 90% survival rate in medium having autoclaved sand + soil + peat in 1:1:1 ratio (Figure 2).

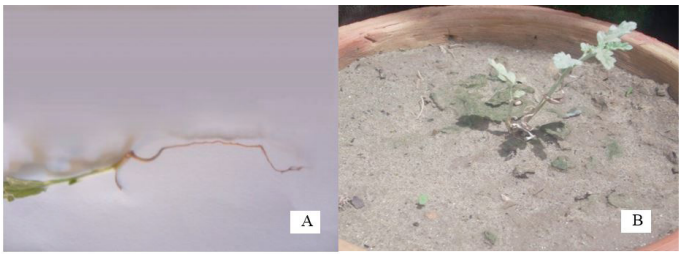


**Figure 2:** *A) Maximum Root length with IBA during invitro rooting, B) Established plant of N. Procumbens during acclimitazation.*

## Conclusion

The micro propagation protocol reported here was characterized with a rapid proliferation of shoots, easy rooting of the micro-shoots and the plantlets were easily acclimatized to the external environment and undergoing normal physiological development. This is highly advantageous for the production of endangered medicinal plant i.e. *Neurada procumbens* L. for conservation and a range of further medicinal and biotechnological applications.

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## Author's Contribution

S.S. Zahra conceived the idea and A. Muhammad facilitate, guided and supervised the experiment. Zareen, S. and M. Asadullah planned and executed the experiment in lab and screen house, also noted the results. M. Asadullah collected the data, did statistical analysis and wrote the manuscript. A. Mehmood helped in writing the manuscript.

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