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Full Length Research Paper

High frequency *in vitro* shoot regeneration of *Momordica balsamina*, an important medicinal and nutritional plant

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A protocol was developed for *in vitro* propagation by multiple shoot induction of *Momordica balsamina* (*Cucurbitaceae*), a climber with high medicinal and nutritional values. High frequencies of multiple shoot regeneration were achieved from auxillary bud of nodal explants. The bud explants were cultured on MS media supplemented with 1.0 mg/L benzyl amino purine (BAP) which stimulated proliferation of the bud meristems to form bud clusters having 6 to 8 co-efficient. The elongated shoots were sub-cultured for rooting on ½ MS media supplemented with 0.3 mg/L NAA and 0.2% activated charcoal. The plantlets raised *in vitro* were acclimatized in green house and successfully transplanted to natural condition with 70% survival. Direct organogenesis of explants and regeneration of *M. balsamina* offer a good opportunity to use tissue culture as a complementary tool for breeding and genetics for other applications.

Key words: *Momordica balsamina,* regeneration, *in vitro* propagation, cucurbitaceae.

INTRODUCTION

Momordica balsamina also known as 'Balsam apple' (African pumpkin), is an important medicinal and nutritional plant of the family Cucurbitaceae. It is an annual to perennial tendril-bearing herb, native to tropical regions of Africa. In India, it occurs naturally in forest, in the rainy season. The leaves, fruits, seeds, and bark of these plants are reported to have various medicinal and nutritional importance (Hassan and Umar, 2006; Bot et al., 2007; Benoit-Vical et al., 2006; Matawalli et al., 2004; Tommasi et al., 1995; Karumi et al., 2003). The fruit pulp extract of *M. balsamina* shows anti-HIV property (Bot et al., 2007). 'Momordins' present in the plant is capable of inhibiting the growth of HIV and other viruses (Ortigao et al., 1992). The leaves and fruit extracts of this plant shows antiplasmodial activity and is being used against malaria as African traditional medicine (Benoit-Vical et al., 2004). The extract of various parts of this plant shows shigellocidal, anti-diarrhoeal, antiseptic, antibacterial, antiviral, antinflammatory and antimicrobial properties (Iwalokun et al., 2001; Banderia et al., 2001; Tommasi et al., 1995; Karumi et al., 2003; Kainyemi et al., 2005; Thakur et al. 2009; Jigam et al., 2004).

M. balsamina is a wonder plant for neutraceutical sciences. The leaves are important source of nutrients having 17 amino acids (Hassan et al., 2006), adequate mineral composition like potassium, magnesium, phosphorus, calcium, sodium, zinc, manganese and iron contributes towards combating the problem of micronutrient deficiencies in soil and high value of protein and fat with low fibre content (Michael and Anthony, 2007; Hassan et al., 2006). The wild vegetable could be

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promoted as a protein supplement for cereal-based diets in poor rural communities and its high potassium content is a good source for the management of hypertension and other cardiovascular conditions.

Due to the high medicinal and nutritional value of this plant, there is need to develop strategies and facilities to checkout the yield potential and development of new technology for commercialization of this plant. The restricted distribution and indiscriminate over exploitation of *M. balsamina* coupled with low seed set, viability, habitat-specific and poor seed germination rate has made its status rare in the wild. Novel propagation techniques like micropropagation can play an important role in the rapid multiplication of elite clones and germplasm conservation of *M. balsamina* plants. The present investigation discusses the tremendous potential for the direct *in vitro* regeneration and multiplication of this potential plant.

MATERIALS AND METHODS

M. balsamina plants were collected from Tropical Forest Region of Jabalpur during monsoon season. These plants were maintained and grown in the Botanical Garden of company campus. Auxillary bud from the actively growing new shoots were excised and used as explant. The explants were rinsed in running tap water for 20 min, cleaned in 10% liquid soap solution for 5 min and then rinsed with double distilled water 2 to 3 times. The explants were surface sterilized with 0.1% mercuric chloride solution for 2 to 3 min in 70% ethanol. Finally, the explants were washed in sterilized double distilled water and cultured on MS medium with vitamins, 3% sucrose, 0.8% agar supplemented with different concentration of cytokinins (0.5 to 5.0) mg/L viz. benzyl amino purine (BAP) and kinetin (Kn) and their combinations (Figure 2) for three weeks. Different concentration of additives like activated charcoal was also added to the media. The medium was adjusted to pH 5.7. All cultures were incubated at 27 ± 2°C under 2000 to 2500 lux light for 16 h/day.

Regenerated shoots were excised and cultured on MS medium with vitamins, 3% sucrose, 0.8% agar and supplemented with different concentration of BAP (0.5 to 5.0 mg/L), Kn (0.5 to 5.0 mg/L) singly and their combinations for multiplication. All the experiments were repeated thrice and had 12 replicates with single explants. Cluster of multiple shoots (1 to 2 shoots) was used as explant in the following experiments. Regenerated and elongated shoots were excised and cultured on MS medium (0 MS, ½ MS and ¼ MS) with vitamins, 3% sucrose, 0.8% agar fortified with auxins viz. indole acetic acid (IAA) and indole butyric acid (IBA) in different concentrations (0.5 to 5.0 mg/L) for rooting. Plantlets with well-developed roots were transferred to green house and cultured in a mixture of peat and sand (2:1). Plantlets were washed thoroughly with distilled water and transferred to pots. Initially, high humidity was maintained by covering with white plastic bags. After 15 days, plastic bags were removed and survival percentage (%) was recorded after one month of transfer.

All experiments were arranged in completely randomized design. Each treatment contained 12 replicates and significant differences among the various treatments were compared.

RESULTS AND DISCUSSION

High frequencies of multiple shoot regeneration were achieved from auxillary buds of nodal explants. The bud explants were cultured on MS media supplemented with different concentration of BAP (Figure 1A). 1.0 mg/L BAP stimulated the proliferation of bud meristems to form bud clusters and the co-efficient reached 6 to 8 (Figure 1B, C). The maximum survival percentage of explant also occurred in the same medium as compared to different concentration of BAP where slow growth was observed in 3 mg/L BAP and no significant results were achieved in 5 mg/L BAP. It is clear that MS medium supplemented with BAP at 1.0 mg/L was most effective in multiplication of shoots but BAP at different concentrations showed high callusing (Figure 1C). Activated charcoal at 0.2% was found to be most effective to inhibit the callus growth. However, BAP at 1.0 mg/L with 0.2% activated charcoal (Figure 1D, E) achieved most significant value of shoot number/explant (7.83). Kn at different concentration did not show any significant rise in shoot number but it did affect elongation of plantlets. The interaction of BAP, Kn, 0.2% activated charcoal with MS at different concentration did not show any significant increase in shoot number. The shoot elongation was prominent (Figure 1F) in MS medium supplemented with 1.0 mg/L BAP and 1.0 mg/L Kn (10.37 cm) as compared to BAP at 5.0 mg/L (2.75 cm) and Kn at 5.0 mg/L (4.43 cm). Nabi et al. (2002) reported regeneration in *Momordica dioica* on MS medium supplemented with 1.0 mg/L BAP and 0.1 mg/L NAA from node shoot tip, leaf and cotyledon explants. Contrary to the studies on *M. balsamina*, multiple shoot regeneration of *Cucumis melo* using shoot tips as explant was found in 2.5 mg/l NAA and 1.0 mg/l BAP (Moreno et al., 1985). The best shoot elongation of *Trichosanthus dioica* Roxb. was observed in MS supplemented with 1.0 mg/L BAP, 0.1 mg/L NAA and 10 mg/L adenine sulphate (Hossain et al., 1997). It is evident from the present studies that combination of auxins promoted excellent shoot elongation and proliferation.

On subculturing, the regenerated shoots of *M. balsamina* in different strengths of MS medium full strengths gave the greatest number of shoots (3.75) multiplication as compared to ½ MS (3.1) and ¼ strength with average shoot length. However, maximum elongation of the shoot was observed in ½ MS when compared to full and ¼ strengths (Figure 3).

Healthy shoots (5 to 6 cm long) were transferred on MS medium supplemented with different concentrations of NAA and IBA to follow multiple shoot elongation. On ½ MS medium supplemented with 0.3 mg/L NAA (Figure 1G and H), maximum number of root/explant (4.75) was observed. Root elongation was simultaneously maximum with root induction in the same medium (Figure 4). Well developed shoots (10 cm) and roots were observed after 30 days. Cultures were subsequently removed from agar medium, washed thoroughly and placed in pots (Figure 1I) containing a mixture of peat and sand (2:1). *In vitro* raised plants were acclimatized in green house and successfully transplanted into field with 80% survival.

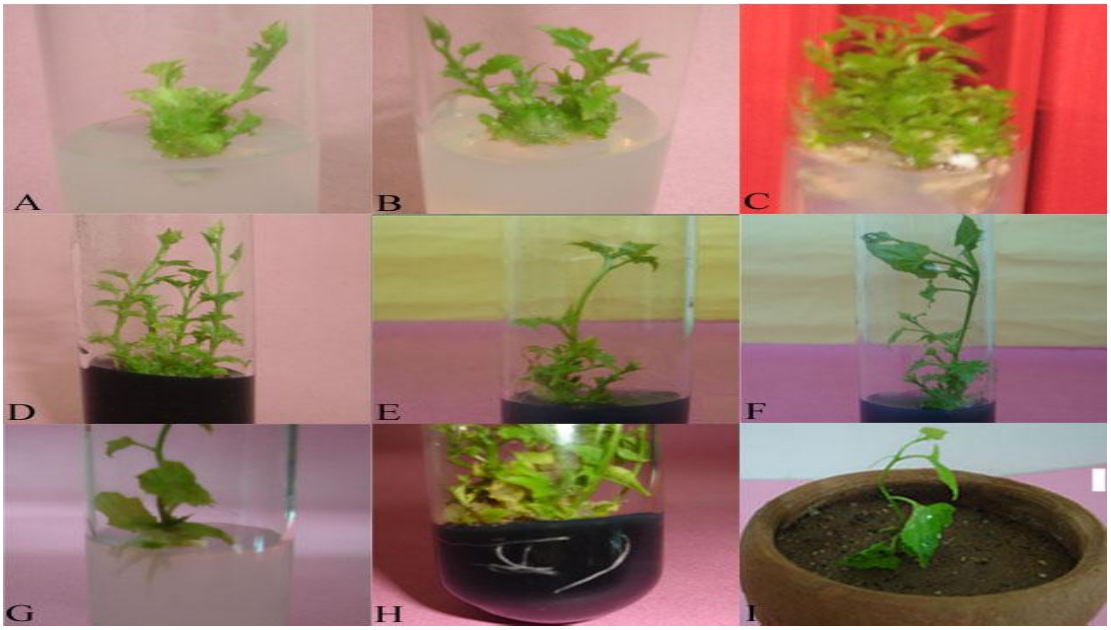

Figure 1. *In vitro* shoot regeneration of *M. balsamina*. A, Shoot proliferation on MS medium supplemented with 1 mg/L BAP after 21 days of incubation; B, shoot multiplication on MS medium supplemented with 1mg/L BAP after 14 days of incubation; C, shoot multiplication and callus formation on medium supplemented with 1 mg/L BAP after 21 days of incubation; D, shoot elongation on MS medium supplemented with 1 mg/L BAP and 0.2% activated charcoal after 30 days of incubation; E, shoot elongation on MS medium supplemented with 1 mg/L BAP, 1 mg/L Kn and 0.2% activated charcoal after 14 days of incubation; F, shoot elongation on MS medium supplemented with 1 mg/L BAP, 1 mg/L Kn and 0.2% activated charcoal after 30 days of incubation; G, rooting observed in ½ MS medium supplemented with 0.3 mg/L NAA; H, rooting observed in ½ MS medium supplemented with 0.3 mg/L NAA and 0.2% activated charcoal; I, acclimatized plants in a pot after 28 days.

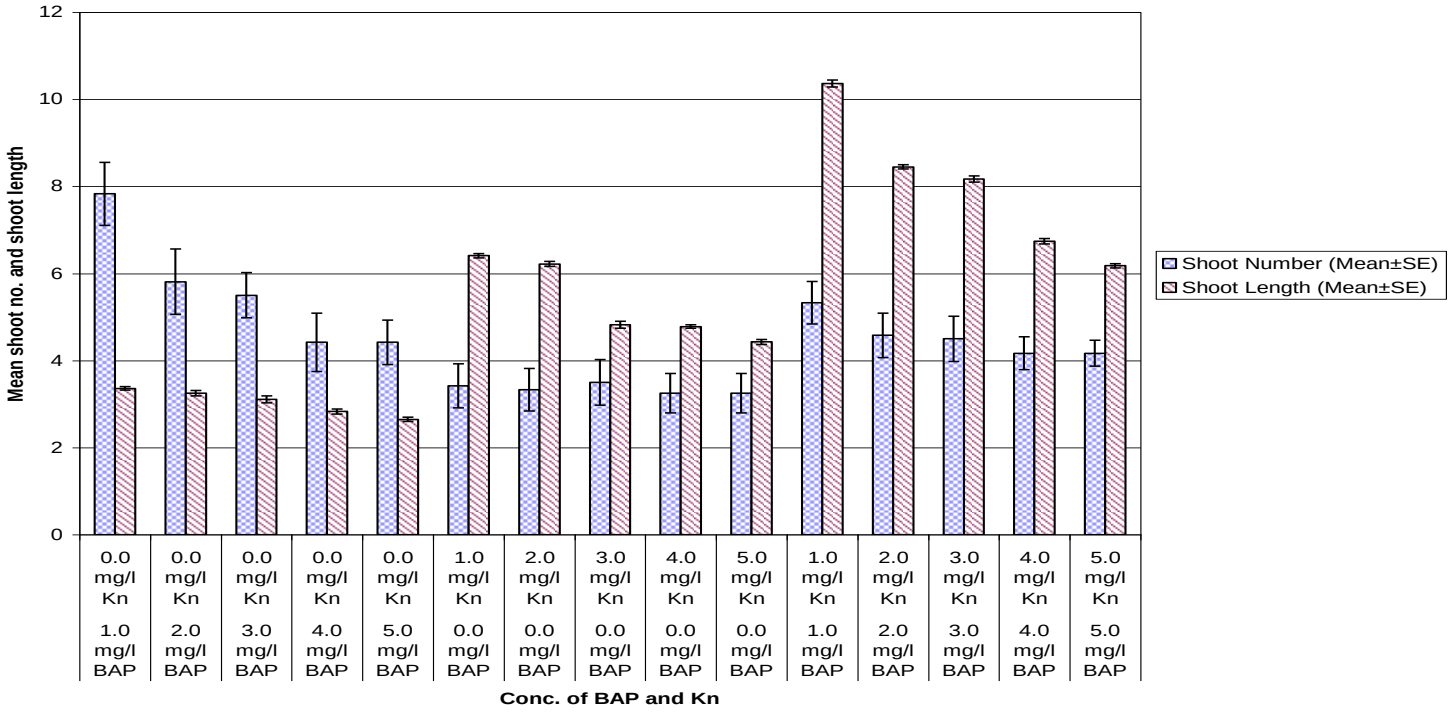

Figure 2. Effect of different concentration of BAP and Kn on shooting of *M. balsamina*.

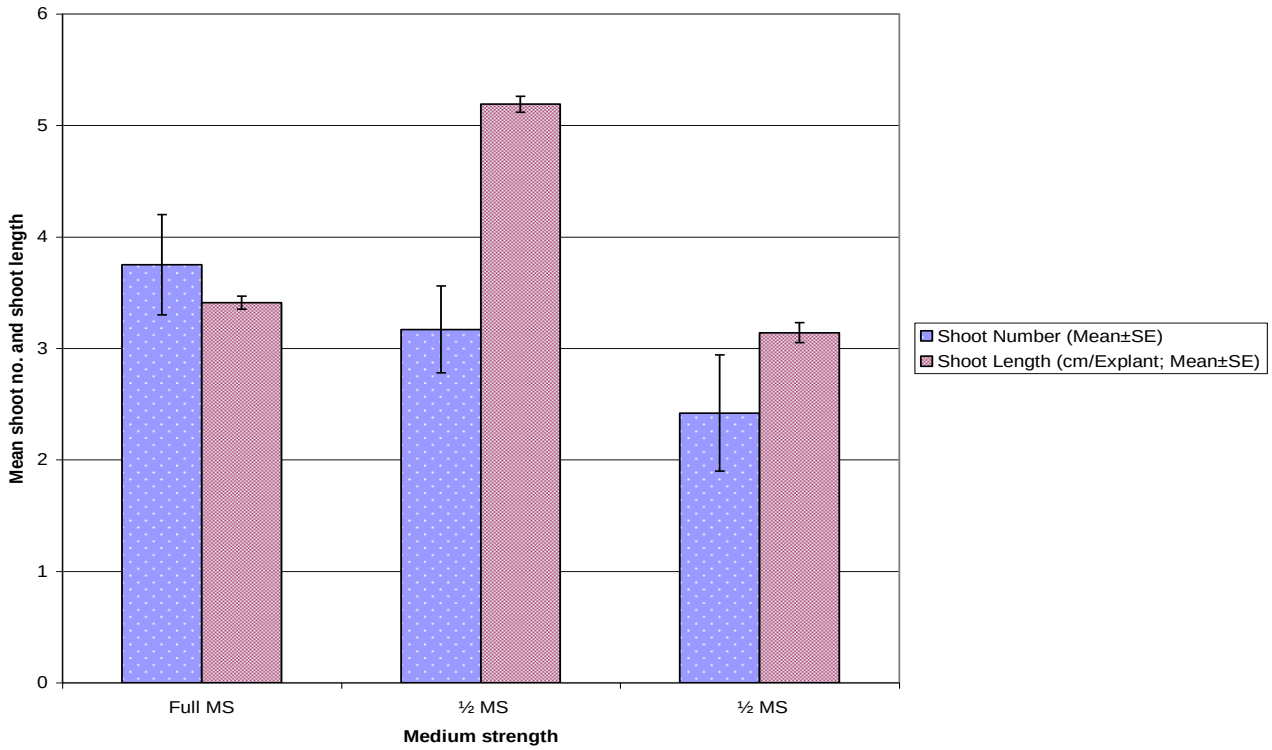

Figure 3. Effect of different strengths of medium on shooting and rooting of *M. balsamina*.

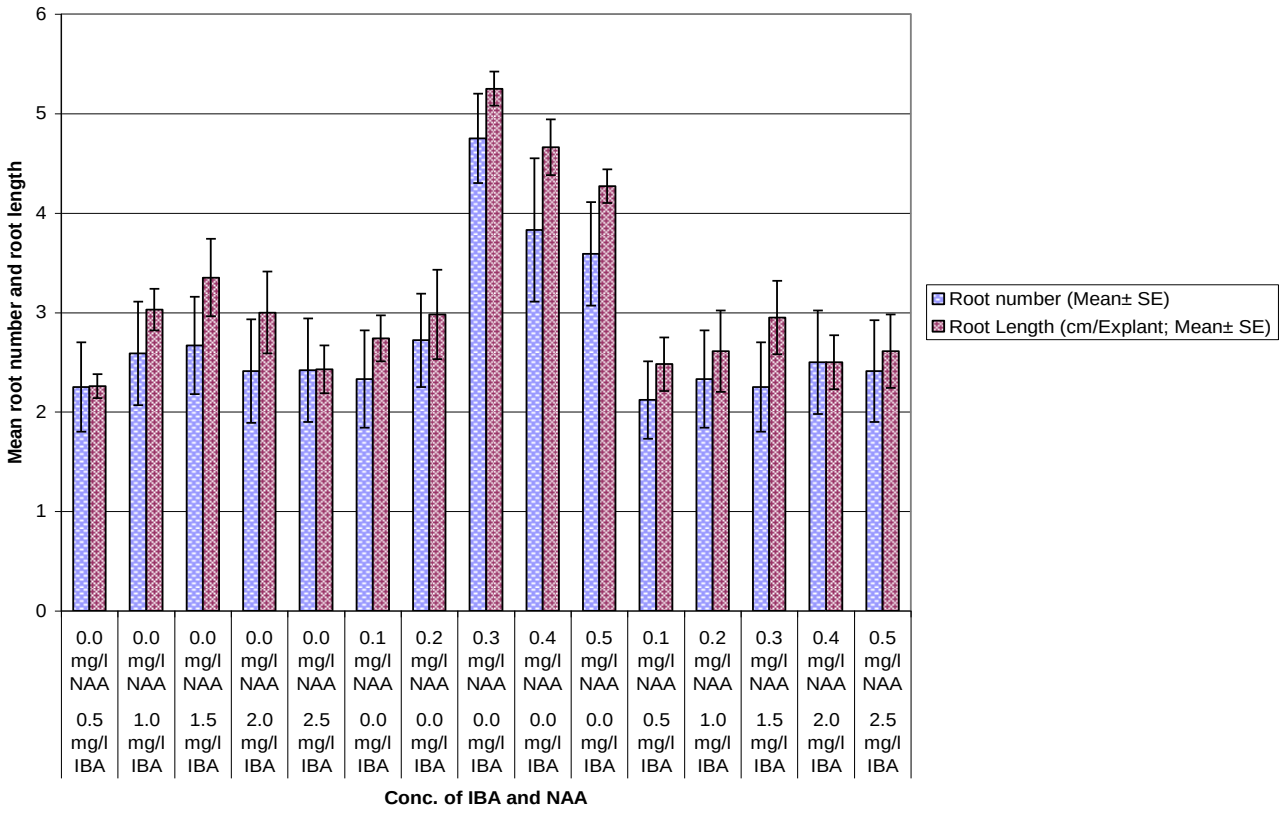

Figure 4. Effect of different concentration of auxins on the rooting of *M. balsamina*.

Conclusion

The results thus, provided evidence that the auxillary shoot buds have the potential to regenerate and act as a source of propagules for conservation of the medicinal and nutritive plant, *M. balsamina*. Further work to isolate bioactive principles and to elucidate the mechanism of medicinal and nutritive properties of the plant extract needs to be determined.

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