International Journal of Recent Biotechnology
Available online at www.ijrbp.net

ISSN: 2322 – 0392
Int. J. Rec. Biotech. **2013,** 1 (1): 1-8

Research Article

IJRB

Effect of Plant Growth Regulators on Proliferation of Multiple Shoots and Callus Induction in Horse gram (*Macrotyloma uniflorum* (Lam.)Verdc.)

Vaishali Bisht[3], Maninder Singh[1], Deepti Shandilya[2], Jitendra Mehta[3]*, Sudershan Rathore[3]

[1]Department of Biotechnology, Shekhwati College, Sikar
[2]Department of Botany, Govt. College, Bundi
[3]Plant Tissue Culture Division, Vital Biotech Research Institute, Kota (Rajasthan)
*Corresponding Author Email: jitendra1242@gmail.com

ABSTRACT

An effective protocol has been developed for Macrotyloma uniflorum. Nodal segments obtained from 15-d-old aseptically grown seedlings were used as explants. MS medium containing 1.0 mg/l BAP was found most suitable for culture initiation. Although shoot multiplication was achieved on MS medium containing BAP and Kn, the maximum number of shoots was obtained with 2.0 mg/l BAP+ 0.5 mg/l Kn. Best rooting response was observed on medium containing quarter strength MS salts, 0.8% agar and 0.5 mg/l IBA. Plantlets were hardened initially in culture room conditions and then transferred to misthouse. Maximum callus induction response was observed on MS medium supplemented with 0.5 mg/l 2,4-D within 4 weeks from leaf petiole.

Keywords:, *Horse gram, Macrotyloma uniflorum, organogenesis, Callus culture*

INTRODUCTION

Tissue culture techniques have largely been integrated in biotechnology that permits the regeneration of plants either as clones or somaclones. The availability of an efficient protocol for *in vitro* regeneration is a prerequisite to harness any biotechnological approach for genetic improvement of crop plants. Development of an effective and reproducible protocol for differentiation of multiple shoots from the callus has a great potential involving *in vitro* mutation breeding and obtaining somaclonal variants. *Macrotyloma uniflorum* (Lam.) Verdc, commonly known as horse gram, is an underexploited multipurpose legume crop of the tropics and subtropics, and is grown mostly under semi-arid conditions.

It is climbing annual herb with slender, slightly hairy stems. Three oblong leaflets, blunt at the apex, the terminal one 1.8 to 2.5 cm long, the lateral ones very unequal sided. Flowers yellow or greenish yellow, one to three on very short pedicels in the axils of the leaves. Pods linear-oblong, slightly curved, sessile, 2.5 to 5.5 cm long, smooth or slightly hairy, six- to eight-seeded, tipped with a persistent style 0.6 cm long.

A summer-growing annual, it requires hot moist weather for maximum growth. Ludlow and Wilson (1970) obtained only 8 percent of the dry matter, 25 percent of the growth rate and 4.5 percent of the leaf area at 20°C as that yielded at 30°C. It is completely intolerant of frost, but usually seeds before the frosts and regenerates in the summer from seed.

It is reported as an excellent source of iron and molybdenum in addition to high content of vegetable proteins. [1] [2] Though there are earlier reports on adventitious proliferation of shoots from the shoot tip and cotyledonary nodal explants[3][4] [5], regeneration from the callus cultures has not been achieved in this taxon. Hence, the present study is an attempt to induce

shoot buds from the callus cultures of shoot apices and cotyledonary nodes of *M. uniflorum*.

MATERIAL AND METHODS

The fruits of *M. uniflorum*. were collected from garden of Herbal Garden Kota, Rajasthan. They were dried. The seeds were surface sterilized subsequently with running tap water, labolin soap solution and finally with 0.1% $HgCl_2$ for 15 min under laminar bench. After rinsing for 5-6 times with autoclaved distilled water, they were inoculated aseptically on to water agar (0.8%) for germination. Cotyledonary and epicotyledonary nodes obtained from 15-d-old seedlings were implanted vertically on to different culture media [Murashige and Skoog (MS)] containing 2.0 mg/l BAP. Different concentrations of BAP and Kn (1.0-5.0 mg/l) were used individually for proliferation of shoots from seedling nodes. Combination of BAP and Kn were used for shoot proliferation. Same experiments were repeated for shoot multiplication.

The medium containing 3% sucrose was solidified with 0.8% agar (Qualigens). The *p*H of the media was adjusted to 5.9+0.02 with 1 N NaOH or 1 N HCl solutions prior to autoclaving. Media poured in culture vessels were steam sterilized by autoclaving at 15 lbs for 15-20 min. The cultures were incubated under controlled conditions of temperature (25±2°C), light (2000-2500 lux for 16 h/d provided by fluorescent tubes) and 60-70% humidity. For each experiment a minimum of 7 replicates were taken and experiments were repeated thrice. Observations were recorded after an interval of 3 wk. Once culture conditions for shoot induction from explants were established, the shoots produced *in vitro* were sub cultured on fresh medium every 3 wk. Different combination of Auxins (0.25 mg/l) (2, 4-D) and Cytokinine (BAP and Kn) (0.1-0.3 mg/l) were used for callus induction from leaf petiole. Rooting of elongated shoots was attempted under *in vitro* conditions. Auxins (IBA) alone in different concentrations (0.5-2.5mg/l) were incorporated in the agar (0.8%) solidified medium containing 1/4 MS salts and 1.0% sucrose. The *in vitro*-rooted plantlets were transferred to culture bottles 1/4th filled with Soilrite composition.

RESULTS AND DISCUSSION

The nodal explants, when inoculated on MS medium containing BAP and Kn in the range 1.0-5.0 mg/l showed enhanced shoot proliferation. BAP at its 1.0 mg/l concentration evoked best response. Shoots after their initial proliferation on medium containing 1.0 mg/l BAP were sub-cultured on same fresh medium after every 21 days. Incorporation of BAP or Kn into MS medium supported multiplication of shoots in culture, BAP proved to be a better choice than Kn and the maximum number of shoots were obtained on its 1.0 mg/l concentration (Table 1, Fig. 1- A, Fig. 2). When BAP was used in combination with Kn a variety of responses were observed (Table 2, 3 Fig. 1-C, Fig. 3). But best response was observed on medium containing 2.0 mg/l BAP + 0.5 mg/l Kn (Average number of shoots 3.31±0.38, shoot length 3.47±0.31).

The full or half strength of MS medium without any PGR was failed to induce rooting of regenerated shoots. However, shoots were capable to induce root when cultured on medium containing auxins. Auxins in different concentration induced rooting when incorporated in the medium containing ¼ of MS salts. The best rooting response, however, was observed on medium containing 0.5 mg/l IBA, where roots measuring 5.31±0.28 cm (average) were formed (Table 4, Fig. 1-E, Fig. 4). *In vitro* rooted plantlets were initially hardened in culture room conditions where leaves expanded. After 3 weeks, the plantlets were shifted to mist house. There was an increase in length of shoots and new leaves emerged which expanded quickly Leaf petiole explants were used for the purpose of callus induction. Highest diameter of callus was observed on MS Medium fortified with 0.5 2,4-D mg/l (callus diameter 3.35 cm) (Table 3, Fig.1 D).

Table-1: Effect of Cytokinin (BAP and Kn) on shoot proliferation from Nodal shoot explant of *Macrotyloma uniflorum*

Hormone Con. (mg/ l)	Hormone Con. (mg/ l)	Response (%)	No. of Shoot/explant (mean±SE)	Shoot length (in cm) (mean±SE)
BAP	**Kn**			
1.0	-	90	4.32±0.38	6.81±0.76
2.0	-	80	3.88±0.41	6.56±0.84
3.0	-	75	3.71±0.56	4.62±0.53
4.0	-	65	2.28±0.26	4.08±0.51
5.0	-	50	1.85±0.50	3.38±0.33
-	1.0	65	2.28±0.26	6.57±0.49
-	2.0	75	3.42±0.39	6.90±0.66
-	3.0	80	3.85±0.37	7.15±0.34
-	4.0	60	2.57±0.20	4.70±0.41
-	5.0	50	1.20±0.31	3.92±0.11

Medium: MS+ additives; mean± SE, n= 7 replicates
Means having the same letter in each Colum, do not different significantly at P< 0.05 (Tukey's test)

Table-2: Interactive effect of Cytokinin (BAP+ Kn) on shoot multiplication by Subculture of shoot clumps of *Macrotyloma uniflorum*

Hormone Con. (mg/ l)	No. of Shoot/explant (mean±SE)	Shooting Response (%)	Shoot length (in cm) (mean±SE)
0.5 Kn+0.5 BAP	2.26±0.34	60	3.30±0.38
0.5 Kn+1.0 BAP	2.98±0.54	85	3.06±0.32
0.5 Kn+2.0 BAP	3.31±0.38	90	3.47±0.31
0.5 Kn+3.0 BAP	1.78±0.37	85	2.06±0.22
0.5 Kn+4.0 BAP	1.26±0.44	83	1.60±0.53

Medium: MS+ additives; mean± SE, n= 7 replicates
Means having the same letter in each Colum, do not different significantly at P< 0.05 (Tukey's test)

Table-3: Effect of different Hormones on Callus proliferation and Morphology of *M. uniflorum*

Hormone Con. (mg/ l)	Callus proliferation Scoring	Response (%)	Callus diameter after 4 weeks subculture (cm)
0.5 2,4-D	++++	90	3.35
1.5 2,4-D	+++	85	3.06
2.0 2,4-D	++	80	2.77
2.5 2,4-D	+++	84	2.56
3.0 2,4-D	++	83	2.60

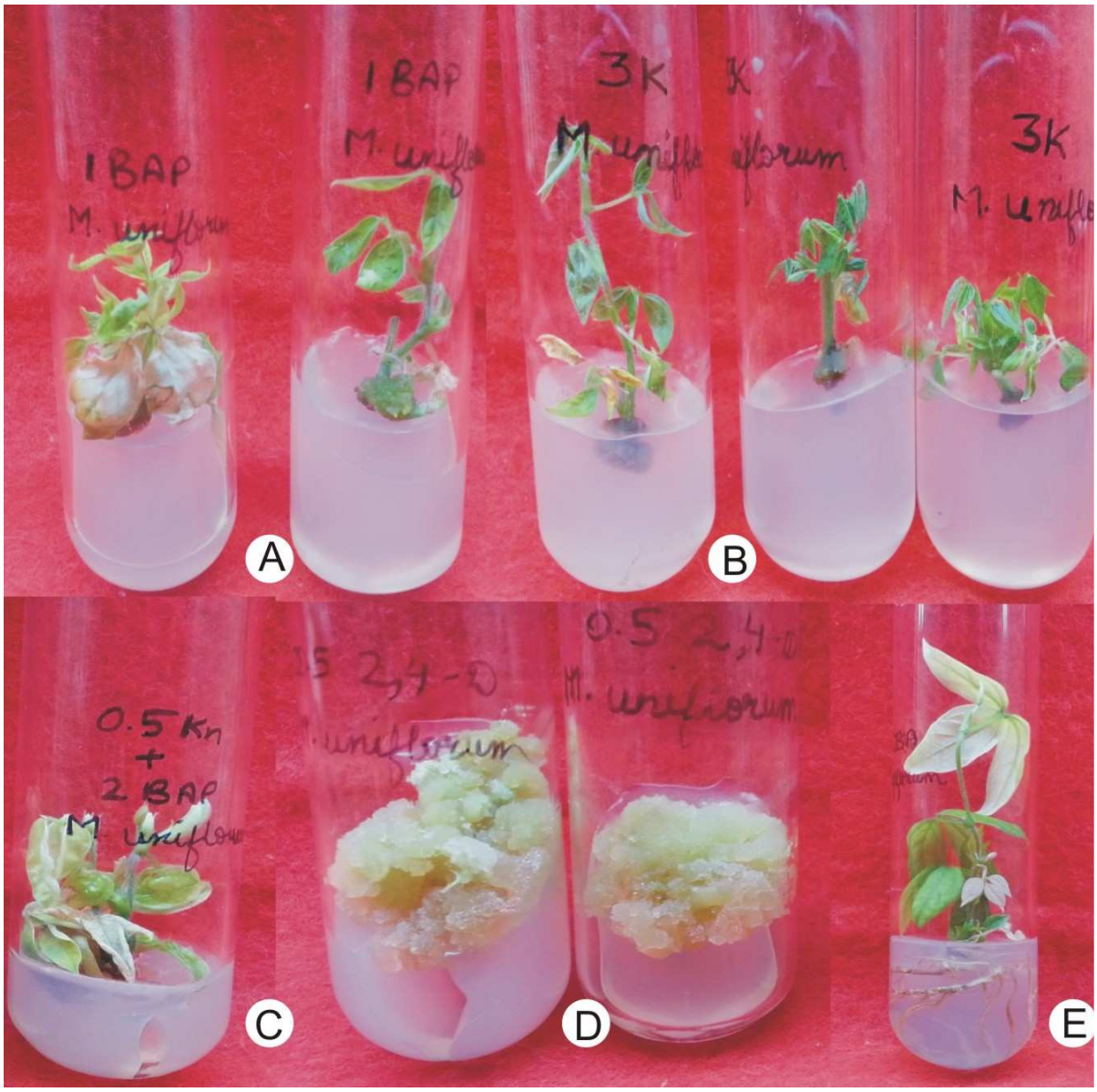
'++++' *Intense,*
'+++' *Moderate,*
'++' *Meager*

Table-4: Effect of Auxin (IBA) on root induction from isolated shoot of *M.uniflorum*

Hormone Con. (mg/ l)	No. of roots/explants (mean±SE)	Rooting Response (%)	Root length (in cm) (mean±SE)
0.5 IBA	3.26±0.34	90	5.31±0.28
1.0 IBA	2.98±0.24	85	4.06±0.32
1.5 IBA	3.11±0.38	80	3.47±0.34
2.0 IBA	2.78±0.17	75	2.06±0.25
2.5 IBA	1.76±0.26	73	2.63±0.53

Medium: MS+ additives; mean± SE, n= 7 replicates
Means having the same letter in each Colum, do not different significantly at P< 0.05 (Tukey's test)

Figure 1 (A-E) Micropropagation of *Macrotyloma uniflorum* from nodal shoot explants



A. Shoot multiplication on MS medium supplemented with 1.0 mg/l BAP, **B.** Shoot multiplication on MS medium supplemented with 3.0 mg/l Kn, **C.** Shoot multiplication on MS medium supplemented with 0.5 mg/l Kn+2.0 mg/l BAP, **D.** Callus induction from leaf petiole on MS medium supplemented with 0.5 mg/l 2,4-D, **E.** *In vitro* root induction on ¼ of MS medium supplemented with 0.5 mg/l IBA

Figure-2: Effect of cytokine (BAP and Kn) on shoot proliferation from nodal shoot explants of *Macrotyloma uniflorum*

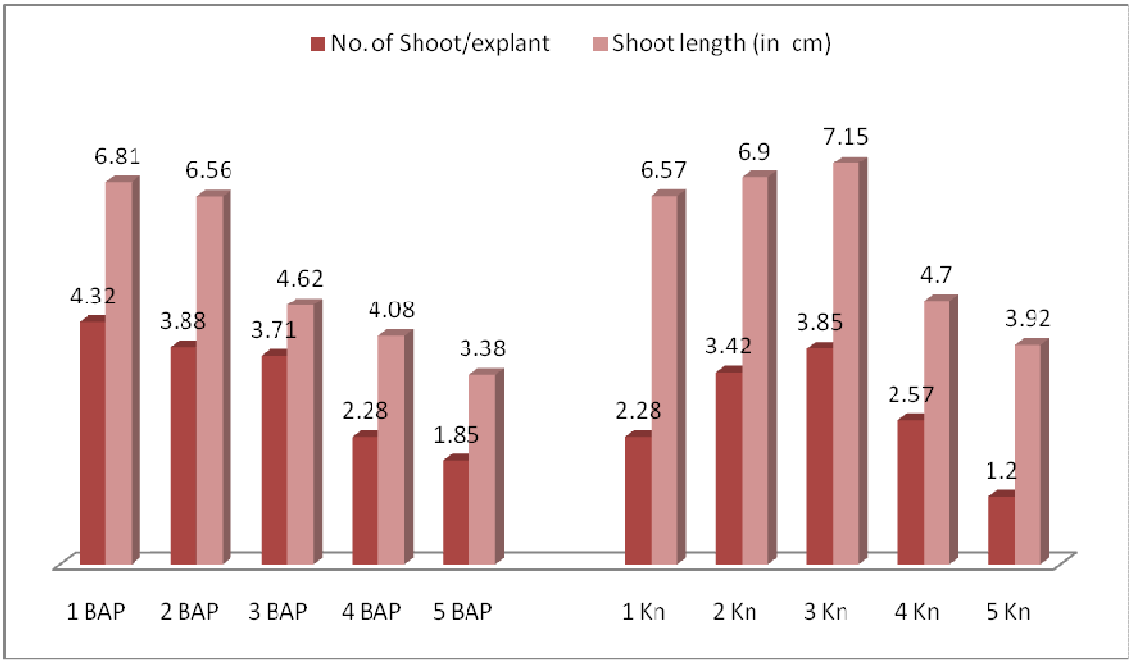


Figure-3: Interactive effect of cytokine (Kn + BAP) on shoot multiplication by subculture of shoot clumps of *Macrotyloma uniflorum*

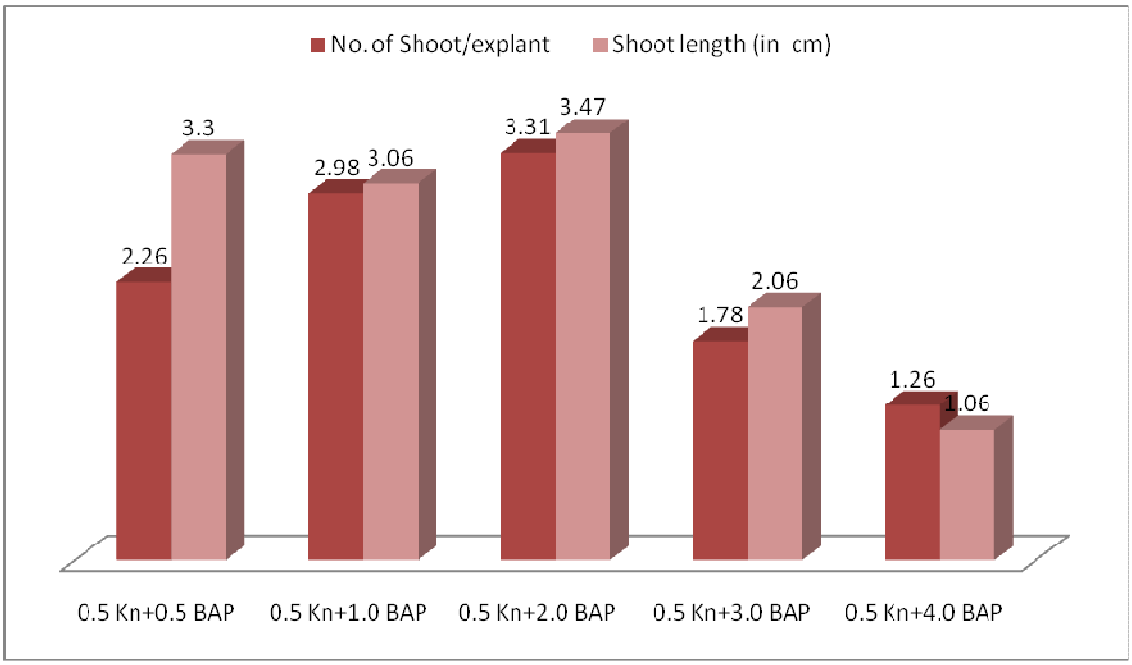
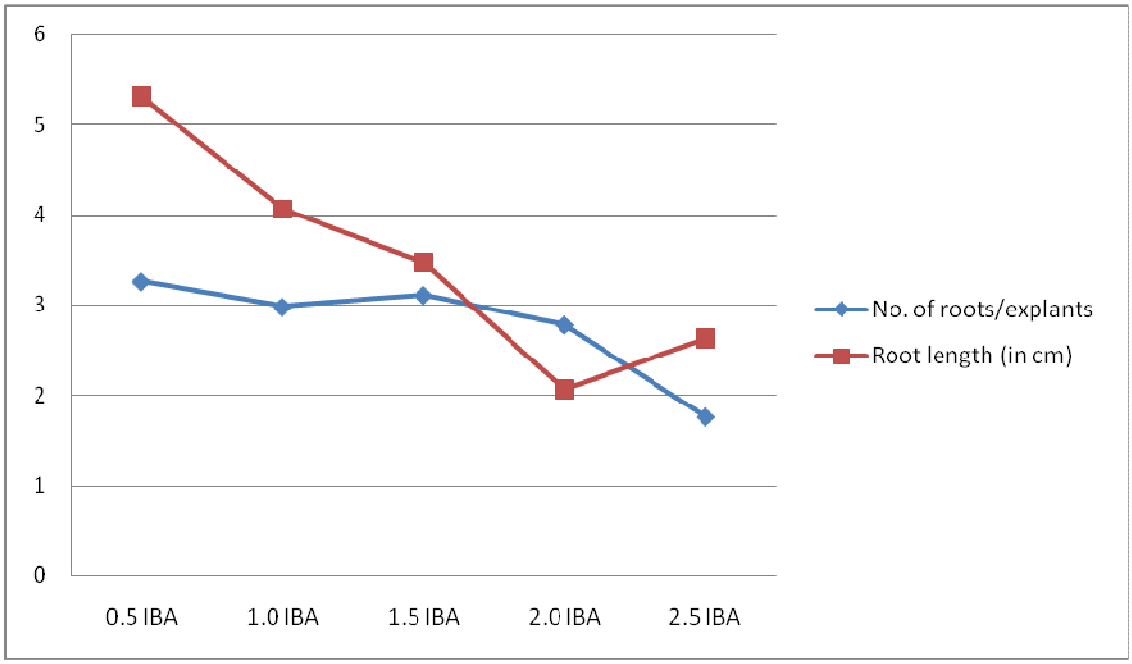


Figure-4: Effect of Auxin (IBA) on root induction from isolated shoot of *Macrotyloma uniflorum*



CONCLUSION

The seedlings derived from explants, being juvenile, are frequently used for micropropagation, as they are easy to establish in culture. In *M. uniflorum*, MS medium containing 1.0 mg/l BAP was the best for culture initiation. We have found that *M. uniflorum*, culture grew better on MS medium in comparison to other media. In *M. uniflorum*, 1.0 mg/l BAP was most suitable for shoot multiplication. We also observed improvement in shoot multiplication by different concentrations of Kn. in medium along with BAP. Best shooting response was observed on media containing 2.0 mg/l BAP+ 0.5 mg/l Kn. IBA (Auxin) has been widely used as root induction hormone under *in vitro* and *in vivo* condition. We also found positive role of IBA during *in vitro* rooting. In *M. uniflorum*, 0.5 mg/l IBA proved to be best for *in vitro* rooting. The *in vitro* rooted plants were hardened first under controlled conditions of culture room and then shifted to misthouse where they exhibited growth with 90% survival. Most responsive callus induction was observed on MS medium supplemented with 0.5 2,4-D mg/l.

Acknowledgement

We are grateful to Plant Tissue Culture Division, Vital Biotech Research Institute, Kota for providing laboratory facilities and also thankful to Jitendra Mehta of Vital Biotech for sincere efforts in writing this research paper. We are also grateful to Ms.Vaishali Bisht, Mr. Maninder Singh, Ms. Deepti Shandilya, Mr. Sudershan Rathore for their continuous team work. We would also like to thank staff members of Vital Biotech Research Institute for encouragement and we would like to thank the reviewers of this paper for their excellent comments.

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