Journal of Crop and Weed, ***9****(1):124-127(2013)*

In vitro regeneration in snake gourd (*Tricosanthes anguina*) from shoot apical meristem and young leaves

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Received: 23-4-2013, Revised: 25-5-2013, Accepted: 30-5-2013

ABSTRACT

Snake (Tricosanthes anguina) gourd is a popular vegetable and medicinal plant in Bangladesh. The present investigation was under taken for in vitro regeneration of snake gourd from shoot apical meristem and juvenile leaves in Murashige and Skoog (1962) medium supplemented with various concentration and combination of BAP, 2,4-D, NAA, IBA and GA_3. Among different concentrations, 1 mg l^{-1}BAP was found for effective callus induction from shoot apical meristem and 2.5 mg l^{-1} 2, 4-D + 0.5 mg l^{-1}BAP from leaves. The highest result for shoot regeneration was observed from meristem derived calli using 1.5 mg l^{-1}BAP + 0.2 mg $l^{-1}GA_3$ and 2.5 BAP + 0.2 mg $l^{-1}GA_3$ from leaves derived calli. In vitro regenerated shoots were rooted well in ½ strength MS with 0.5 mg l^{-1}NAA and well rooted plantlets were successfully established in pot soil with 80% survival rate.

Key words*:* in vitro, juvenile leaves, regeneration, snake gourd, shoot apical meristem

Snake gourd (*Tricosanthes anguina*) is a well known vegetable as well as important for its medicinal value. It is an annual climber belonging to the family cucurbitaceae. *Tricosanthes anguina* is used in the treatment of headache, alopecia, fever, abdominal tumors, bilious, abortifacient, vermifuge, stomachic, refrigerant, purgative, malaria, laxative, hydragogue, hemagglutinant, emetic, cathartic, bronchitis and anthelmintic (Nadkani, 2002). Meristem tissue is the suitable part of a plant which can be used in tissue technique for frequently regeneration as it is the main region of somatic cell division and a main part of *In vitro* virus free plant production. Meristem culture is a unique technique to produce plant to free from other pathogens including viroides, mycoplasma, bacteria and fungi. Juvenile leaf of a plant is another important part for *in vitro* regeneration. Many workers have reported on *in vitro* regeneration from leaves of different plants i.e., Thakur and Srivastava (2006) in Himalayan poplar. Gopi and Ponmurugan (2006) in *Ocimum basilicum* L., Mohanty and Ghosh (1988) in *Hordeum vulgare*, Lata *et al*. (2002) in Black Cohosh. But there is no report yet published on snake gourd from meristem and leaves. In this investigation, therefore, attempt was under taken to develop a suitable protocol for *in vitro* regeneration of snake gourd from young leaves and shoot apical meristem.

MATERIALS AND METHODS

Shoot tips for isolation of meristem and young leaves snake gourd were collected from juvenile *in vivo* grown plants of *Trichosanthes anguina*. These explants were taken into a conical flask and thoroughly washed under running tap water for 20 minutes followed by distilled water containing 1% savlon (v/v) and two drops of tween-80 for 10 minutes for removing loose contaminates attached. Then the explants were washed by distilled water for several times. Subsequently, the explants were transferred to laminar airflow cabinet. Washed shoot tips were treated with 0.05% $HgCl_2$ for 2.5 minutes and leaves were treated for 1 minute at the same concentration of $HgCl_2$ for surface sterilization. Then meristems of 2.50-3.0 mm size with two leaf primordea were isolated from shoot tips under 4X zoom stereomicroscope and quickly culture on Paper Bridge in liquid medium. On the other hand leaves were cut to in small pieces and transferred on semisolid medium. MS media (Murashige and Skoog, 1962) containing sucrose 3% and different concentrations and combinations of BAP (0.5-4.5 mg l^{-1}), GA_3 (0.1-0.3 mg l^{-1}), 2.4-D (1.0-3.5) mg l^{-1} were used for callus induction and regeneration from callus. The pH of the medium was adjusted to 5.70. Then the medium (10 ml) was dispensed into test tubes capped with non-absorbent cotton plugs and stream sterilized by autoclaving the medium at 121°C for 21 minutes at 1.0 kg cm^{-2} pressure. After inoculation, cultures were taken in dark chamber for 2 days and then incubated at 25 ± 2°C under the warm fluorescent light intensity varied from 2000-3000 lux in growth chamber. After 10 days of bridge culture, meristems were transfer in semisolid medium for induction of callus. Several passes of subcultures were done for callus development, formation of multiple shoot buds (MSBs) and shoot elongation from both types of explants. Elongated shoots were rooted on transferring in ½ strength MS media with various concentrations of IBA (0.1 and 2.5 mg l^{-1}) and NAA (0.5-1.5 mg l^{-1}).

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RESULTS AND DISCUSSION

In vitro regeneration of Snake gourd from shoot apical meristem and juvenile leaves using Murashige and Skoog (1962) medium supplemented with different concentrations and combinations of BAP, NAA, 2,4-D, IBA and GA_3 for different purposes such as meristem development, callus induction from meristem, callus induction from leaves, regeneration from both types of callus and root induction. In meristem culture, at first primary establishment of meristems were done on Paper Bridge in liquid MS medium without growth regulators. Ahmed *et al*. (2000) reported the same result in tomato. After subculture in semisolid MS media, callus induction was found using different concentrations and combinations of BAP (0.5-2.5 mg l^{-1}) and NAA (0.5-1.5 mg l^{-1}) either singly or in combinations (table-1).

Table 1: Effects of BAP and NAA in singly or in combinations on callus formation from shoot apical meristem of snake gourd

MS With growth regulators	Explants produced callus (%)	Callus type
BAP		
0	-	-
0.5	30	CG
1.0	90*	CG
1.5	80	CG
2.0	65	CG
2.5	50	CG
BAP + NAA		
0.5 + 0.5	35	CLG
1.0 + 0.5	45	CLG
1.0 + 1.0	60	CC
1.5 + 1.0	70	CC
1.5 + 1.5	55	CC

Note:= Highest percentage of explants formed callus*

Table 2: Effects of BAP, NAA and 2, 4-D on callus formation from young snake gourd leaves

Ms Growth regulators (mg l^{-1})	Explants induced callus (%)	Callus type	Degree of callus formation
2,4-D + BAP			
1 + 0.1	20	CLG	+
1.5 + 0.2	50	CLG	+
2 + 0.2	60	CG	++
2.5 + 0.5	85	CG	+++
3 + 0.5	70	CG	++
3.5 + 0.2	65	CG	++
BAP + NAA			
1 + 0.1	15	LB	+
1.5 + 0.2	40	LB	+
2 + 0.2	45	BF	++
3 + 0.3	50	BF	++
3.5 + 0.3	65	LYF	++
4 + 0.2	70	YF	+++
4.5 + 0.3	60	YF	++

Note: + = Poor callus, ++= Moderate callus, +++ = Good callus, CG= Compact green, CLG= Compact light green, LB= Light brown, BF= Brownish friable, LYF= Light yellowish friable, YF= Yellowish friable

Among the different concentrations 1.0 mg l^{-1}BAP gave good result and produced compact green callus (Fig. 1), but the combinations of BAP with NAA produced creamy and light green callus which were not suitable for regeneration. Indirect shoot multiplication from meristem derived calli was observed in MS fortified with different concentrations and combinations of growth regulators BAP (0.5-3.0 mg l^{-1}) and GA_3 (0.1-0.2 mg l^{-1}) (table-3). Best result of shoot multiplication was obtained from 1.5 mg l^{-1} BAP and 0.2 mg l^{-1} GA_3 (Fig. 1) which is similar to Haque *et al.* (2010) in pumpkin.

Table 3: Effects of BAP and GA_3 singly or in combination on regeneration of shoots from meristem derived calli

MS with growth regulators (mg l^{-1})	Callus produced shoots (%)	Mean no. of shoots after days	
		14	21
BAP			
0.5	10	1	1.20
1	30	1.50	1.80
1.5	40	1.90	2.40
BAP + GA_3			
0.5 + 0.1	20	1.70	2.10
1 + 0.2	70	3.20	4.30
1.5 + 0.2	80	4.60	5.50
2 + 0.2	60	3.80	4.20
3 + 0.2	50	2.70	3.50

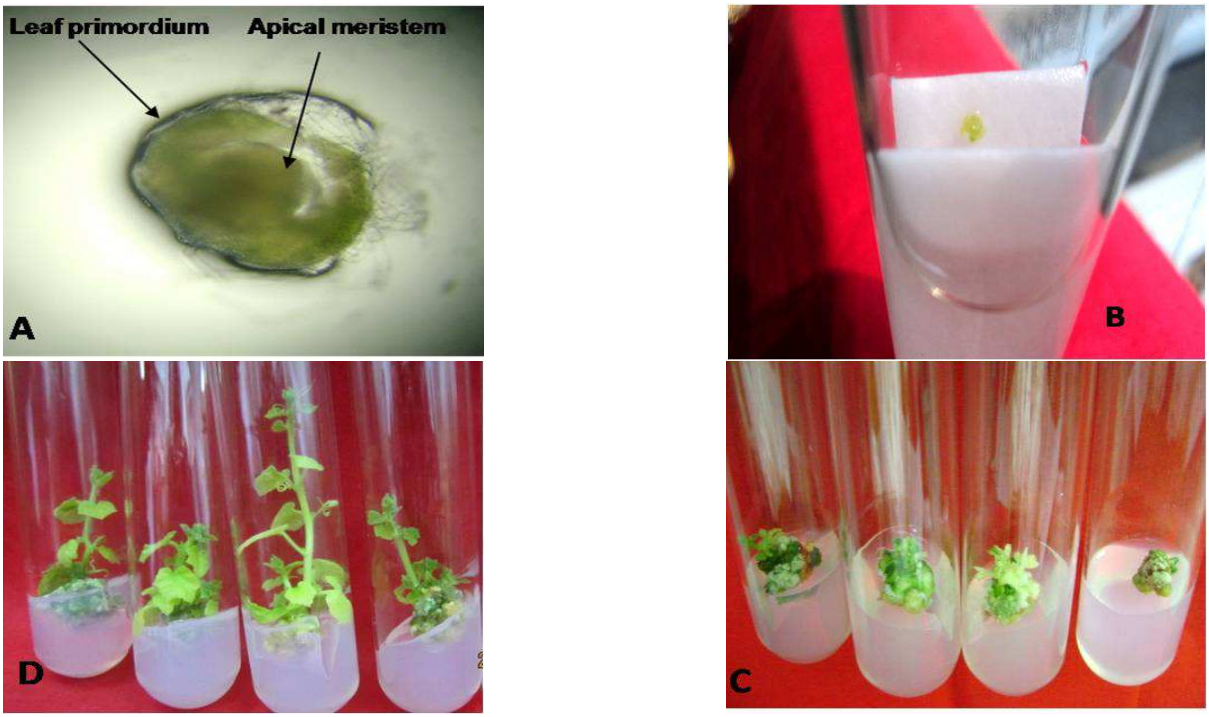

Fig. 1: A- Isolated meristem from shoot tip, B- Development of meristem on paper bridge in liquid MS medium, C- Callus development and shoot bud initiation in MS medium containing 1.0 mg l^{-1} BAP, D- Shoot elongation in MS medium containing 1.5 mg l^{-1} BAP + 0.2 mg l^{-1} GA_3

Fig. 2: E- Callus induction from leaf in MS medium with 2.5 mg l^{-1} 2, 4-D + 0.5 mg l^{-1} BAP, F&G - Shoots bud formation and shoot initiation and elongation in MS with 2.5 mg l^{-1} BAP + 0.2 mg l^{-1} GA_3, H- Root induction in ½ MS + 0.7 mg l^{-1} NAA

However, Huda and Sikdar (2006) reported on meristem culture of bitter gourd using KIN with GA_3. Leaf explants were cultured on MS media with various levels of 2,4-D (1-3.5 mg l^{-1}) and NAA (0.1-0.3 mg l^{-1}) in combination with BAP (0.1-4.5 mg l^{-1}) for callus induction (table-2) and various concentrations of BAP were used in different combinations with GA_3 for shoot regeneration (table-4).

Table 4: Effects of BAP and GA_3 singly or in combinations on shoots multiplication from callus of leaves

MS with growth regulators (mg l^{-1})	Callus produced shoots (%)	Mean no. of shoots after days	
		14	21
BAP			
1	10	1.20	1.60
1.5	10	1.50	2.00
2	20	2.40	2.80
BAP + GA_3			
1.5 + 0.1	10	2.70	3.60
2 + 0.2	25	3.20	4.30
2.5 + 0.2	60	4.40	5.20
3 + 0.2	60	3.80	4.20
3.5 + 0.2	55	3.00	3.50

During this investigation among the different concentrations, 2.5 mg l^{-1} 2,4-D and 0.5 mg l^{-1} BAP were found highly effective for callus induction (Fig. 2) and 2.5 mg l^{-1} BAP + 0.2 mg l^{-1} GA_3 was better for shoots formation from callus (Fig. 2) via organogenesis. Sultana *et al*. (2004) reported that the highest frequency of callus induction (100%) was recorded on medium containing only 2.5 mg l^{-1} 2, 4-D in leaf explants of watermelon. Only BAP was found to be effective for callus induction in leaf explants of pointed gourd (Komal, 2011). *In vitro* developed microshoots were subcultured for rooting on ½ MS with IBA (0.1-2.5 mg l^{-1}) and NAA (0.3-1 mg l^{-1}) separately. Out of the different concentrations of these two growth regulators, 0.5 mg l^{-1} IBA and 0.7 mg l^{-1} NAA were suitable combinations (Fig. 2) for root induction. Ahmed and Anis (2005) achieved good result for root induction in *Cucumis sativus* using similar hormone in ½ MS. But Huda and Sikdar (2006) observed better rooting of *Momordica charantia* L. in the combination of 0.5 mg l^{-1} IBA and 0.1 mg l^{-1} NAA. After 30 days of sub culture, well developed rooting micro plants were removed from agar medium and washed carefully by distilled water. About 90% of the rooted plantlets were acclimatized after hardening and successfully transferred to the field with 80% survival rate.

ACKNOWLEDGEMENTS

Authors are grateful to Professor Joarder DNA and Chromosome Research Laboratory, Department of Genetic Engineering and Biotechnology, Uniersity of Rajshahi, Rajshahi-6205, Bangladesh and USDA and MoE, Bangladesh for their financial support.

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