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REGENERATION OF PLANTLETS FROM NODAL CULTURES OF *MOMORDICA DIOICA* ROXB.

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ABSTRACT

The Callus cultures were raised from nodal explants of *Momordica dioica* Roxb. and the callus was inoculated on MS medium supplemented with different concentrational combinations of 2,4-D, IAA and BAP. Good amount of compact and green callus from nodal cultures was achieved on MS [1] medium fortified with 2.0 mg/l 2,4-D + 1.0mg/l BAP. Regeneration of plantlets were achieved on MS medium fortified with 2.0mg/l BAP + 1.0 mg/l IAA. High frequency of regeneration of plantlets was achieved on the same medium after the sub culture. Later such plantlets were grown for two weeks and rooted on MS +3.0mg/l IBA. The rooted plantlets were grown in green house for hardening and transferred to soil.

KEYWORDS :Regeneration, Plantlets, Nodal cuttings, Shoot bud differentiation, Micropropagation;

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INTRODUCTION

M. dioica Roxb. is commonly known as Kakrol or Teasle gourd. It is a significant medicinal plant originated in Indo-Malayan region [2]. It is one of the most nutritive cucurbit vegetable that holds a coverted position in the market during rainy season. The fruit is rich in proteins, carotenoids, carbohydrates and vitamin-C [3&4]. Traditionally it is being used for treating eye diseases, poisoning and fever [5]. Fruits, leaves, tuberous roots are used for diabetis. The plant was reported to posses antibacterial, Jaundice, bleeding Piles curing properties [6]. The seed germination is low, probably due to presence of bulk cell wall and seeds are dormant for many days [3]. It is not possible to predict the sex of plant produced from seeds. Successful application of plant development for plant improvement requires the development of an efficient shoot regeneration from cultured tissues.

MATERIAL & METHODS

Momordica dioica Roxb. plants were collected from different forest areas of Warangal district during rainy season and planted in research field located in our department. Different explants of the plant were taken for tissue culture studies. Nodal cuttings, internodes, petioles and leaves were sterilized and inoculated on MS medium supplemented with BAP 1.0, 1.5, 2.0, 2.5, 3.0 mg / l and , IAA with 0.5 mg/l – 1.0 mg/l (table-1). Among all the explants nodal cuttings and leaves shown better results for both callus induction and regeneration of plantlets. All the cultures were maintained at 25 ± 2ºC, varying photo period 16 hrs, 12 hrs, 8 hrs were tried to test on regeneration. Cultures were grown under florescent light on successive subculture and small buds were proliferate from the callus with a photo period of 16 hours. Small plantlets were separated and transferred to MS basal medium for further development. The developed plantlets were rooted on MS medium supplemented with 3.0 mg/l IBA and then to green house.

Table 1

Effect of different concentrational combinations of 2,4-D and BAP in the morphogenetic response of nodal explants of Momordica dioica. Roxb.

S.No.	MS medium + 2,4-D + BAP (mg/l)	Morphogenetic response
1.	1.0 + 0.5	Low proliferation of friable callus
2.	1.5 + 0.5	Low proliferation of friable callus
3.	2.0 + 0.5	Moderate proliferation of friable callus
4.	2.5 + 0.5	Moderate proliferation of friable callus
5.	3.0 + 0.5	Low proliferation of friable callus
6.	1.0 + 1.0	Low proliferation of campact callus
7.	1.5 + 1.0	Moderate proliferation of campact callus
8.	2.0 + 1.0	High proliferation of campact callus
9.	2.5 + 1.0	Moderate proliferation of campact callus
10.	3.0 + 1.0	Low proliferation of campact callus

Data was scored at end of the four weeks after the subculture on the regeneration medium.

Table 2
Effect of different concentrational combinations of BAP and IAA in the morphogenetic response of nodal explants of Momordica dioica. Roxb.

S.No.	MS medium + BAP + IAA (mg/l)	Morphogenetic response
1.	1.0 + 0.5	Proliferation of friable callus
2.	1.5 + 0.5	Proliferation of friable callus
3.	2.0 + 0.5	Callus and plantlet formation (2 – 4 shoots)
4.	2.5 + 0.5	Callus and plantlet formation (4 – 8 shoots)
5.	3.0 + 0.5	Proliferation of callus
6.	1.0 + 1.0	Proliferation of callus
7.	1.5 + 1.0	Greening of callus and regeneration (4 – 8 shoots)
8.	2.0 + 1.0	High frequency of regeneration (8 – 12 shoots)
9.	2.5 + 1.0	Moderate formation of plantlets (4 – 6 shoots)
10.	3.0 + 1.0	Moderate formation of plantlets (4 – 6 shoots)

Data was scored at end of the four weeks after the subculture on the regeneration medium.

RESULTS AND DISCUSSIONS

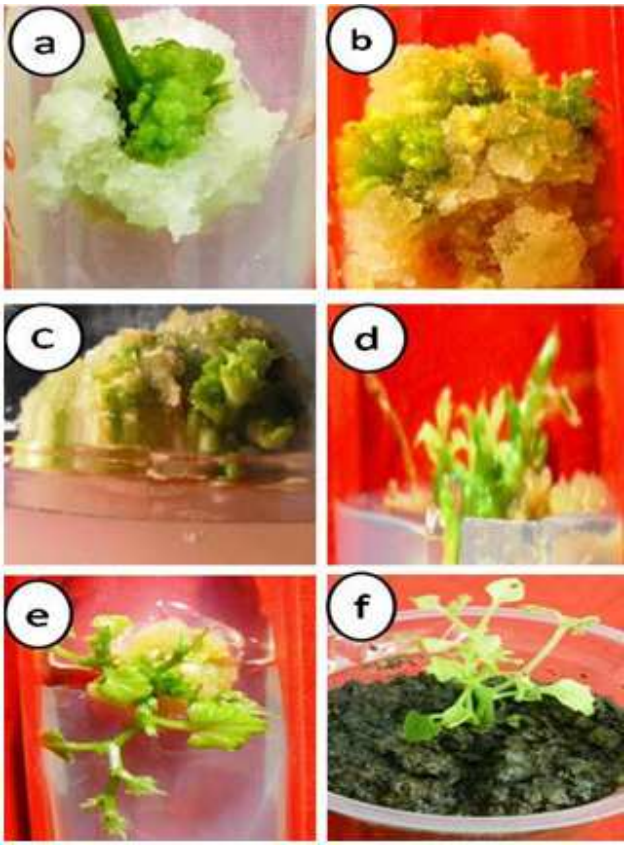
The efficiency of callus induction was more on MS + 2.0 mg/l 2,4-D + 1.0 mg/l BAP (Fig a,b). As the concentration of BAP was increased the shoot bud differentiation was promoted after four weeks of sub culture. The frequency of shoot bud regeneration from the callus was maximum on MS medium supplemented with MS + 2.0 mg/l BAP +1.0 mg/l IAA (Fig c,d). Sucrose played a prominent role in this regeneration process. 3.5% sucrose was proved as ideal for higher rate of regeneration. The frequency of regeneration was enhanced on the same medium after next subculture (Fig e, f). The results of the present studies coinciding with the findings of Hoque *et al*, (1995) [7]. They found that 1.5 mg/l BAP and 0.1 mg/l NAA was more suitable for adventitious multiple shoot formation in the teasle gourd, where as in this experiment 2.0 mg/l BAP + 1.0 mg/l IAA was recorded to be the best for the regeneration of shoot buds from nodal explants after successive subcultures, where as cotyledons are not suitable for the production of plantlets. However plantlets were regenerated from immature embryos of the seeds by Hoque *et al*, (2007) [8], could not achieve regeneration from node of matured plant. Our results were nearer to the findings of Houque *et al*, (1995) [7] investigation and supporting for the fastest and effective multiplication of plantlets in short duration.

Earlier studies have demonstrated the additive effect of BAP and Kn inducing multiple shoot from nodal explants [7]. The concentration of BAP when raised (from 1.0mg/l to 2.0mg/l), the light green callus turned to green organogenic callus (Fig c, d) and proliferated to planlets (Fig e), earlier reports are similar as on cotyledon explants of *Momordica dioica* [9] and hypocotyls in *cucumis melo* cv pusa [2], using leaf,stem and cotyledonary explants of *Momordica charantia* by singh *et al*, (1990) [2]. BAP is generally regarded as the most effective cytokinin for shoot differentiation. The most of the Cucurbitaceae members produced shoots from the callus culture in combination of auxin and cytokinins, Nabi *et al*, (2002) [10] reported shoot induction in *Momordica dioica* and Sultana and Barimiah (2003) [11] in *Momordica charantia*. In our investigation we could achieve high frequency of regeneration of plantlets on MS + 2.0mg/l BAP + 1.0 mg/l IAA. The present investigations easily reveals that the auxin (IAA) lower concentration along with cytokinin is suitable for regeneration and IBA is efficient for the induction rooting from

plantlets. Successful micropropagation depends on the production of healthy and strong root system. *In vitro* plantlet was rooted on MS medium supplemented with IBA and IAA. Rooting was found only with IBA (3.0 mg/l) after 12 days of culture (Fig f).The present investigation revealed that auxin IBA had better effect for the induction of roots. Agarwal *et al,* (2004) [12] obtained similar results in *M. Charantia & M. dioica*.

Figure

Induction of Callus and Regeneration of Plantlets in Momordica dioica Roxb.



(a, b) Induction of callus from nodes of Momordica dioica Roxb. on MS + 2.0 mg/l 2,4-D + 1.0 mg/l BAP. (c, d) Regeneration of shoot buds on MS + 2.0 mg/l BAP + 1.0mg/l IAA. (e) Regeneration of plantlets after subculture. (f) Rooted plant under hardening process.

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