



DEVELOPMENT OF PROTOCOL FOR MICRO PROPAGATION OF GYNOECIOUS BITTER GOURD (*MOMORDICA CHARANTIA* L).

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ABSTRACT: Bitter gourd (*Momordica charantia*.L) is an important cucurbitaceous crop. The occurrence of gynaecium in bitter gourd is very rare and generally they have monoecious sex form. Recent identification of some gynoecious lines showed their advantages in hybrid breeding programme. Our present investigation outlines the *in vitro* propagation of monoecious line of bitter gourd. The auxiliary bud and apical bud of identified gynoecious lines of bitter gourd were cultured on MS medium for initiation. After 3rd subculture stage the average number of shoots and shoot length of bitter gourd was recorded 3.4 and 2.7 cm in auxiliary bud (AXBG) and 2.8 and 2.1 cm in apical bud bitter gourd (APBG) explants respectively. Maximum shoot length was obtained in ABG-6 medium. (½ MS supplemented with 0.5 mg/ml BAP), during the third subculture stage. Rooting was best observed in RBG-7 medium (½ MS supplemented with 1.0mg/ml IBA). When transferred to the primary hardening stage 44% of the plants were survived successfully. For secondary hardening, primary hardened plants were transferred to polybags containing soil, FYM and sand (3:2:1), 50 % of the plants were survived.

Key words: Apical bud, Auxiliary bud, BAP (6- Benzyl amino purine), micro propagation, hardening.

INTRODUCTION

Bitter gourd, balsam pear, bitter melon or bitter cucumber is an important vegetable crop of family cucurbitaceae. Fruits of bitter gourd are consumed across the world as a vegetable and are well known for its medicinal properties such as antidiabetic and others [18]. A triterpenoid compound called 'Charantin' present in bitter gourd is a potential substance used for the treatment of diabetes to lower blood sugar level [9, 6, and 13]. Recent studies also confirmed that it increases body's resistance against various pathogenic infections [3]. The fruit being rich in Vitamin A, Vitamin C, thymine, riboflavin and minerals like iron, is considered as the most nutritive among cucurbits [11]. In cucurbits, several flowering habits (sex forms) have been described like andromonoecious, androgynocious, gynomonoecious monoecious, gynoecious, etc. [7, 17]. Gynoecious sex form has been also reported in recent past from India [2]. The subsequent generation developed by using gynoecious lines as one parent shows positive impact on hybrid production in terms of fruit yield and earliness [5]. Recent studies on gene inheritance revealed that this trait is under the control of a single recessive gene called *gy-1* [15, 4]. In melon (*Cucumis melon* L.), a single recessive gene for gynomonoecious condition has also been reported (mostly pistillate flowers and a few hermaphrodite or perfect flowers) [14]. However, in bitter gourd, occurrence of gynaecium is very rare. Hybrid in bitter gourd is generally produced by hand emasculation and hand pollination technique, both is very costly and labour intensive. Furthermore, the number of hybrid seeds per fruit of bitter gourd is also very less. Therefore, gynoecious line can be used to produce hybrids economically in bitter gourd. An inheritance of gynaecium (especially femaleness) has been well documented in cucumber too, and was used for effective hybrid seed production at commercial level [8, 16]. Present study was conducted to develop *in vitro* protocol for micropropagation of gynoecious bitter gourd for use in hybrid seed production at commercial level.

MATERIAL AND METHODS

About 5 to 6 centimetres in length of auxiliary and apical bud of bitter gourd were collected in the month of November-2013 as explants for *in vitro* initiation. The mother plant (Fig. 1.0) was identified in one of the experimental plots of at Aditya biotech Lab & Research Pvt. Ltd. Raipur, Chhattisgarh, India.

Pre-treatment

Auxiliary and apical buds of bitter gourd were pre treated by dipping in 5% Sodium Hypochlorite solution at the time of collection. Further the explants were dipped in fungicide (bavistin) Ampicillin, solution having 0.1 % and 0.5 % concentration respectively followed by washing with sterile distilled water 2-3 times. A final treatment with 0.2 % HgCl₂ (W/V) was given for five minutes after which the plants were thoroughly washed three times with sterile distilled water and subjected to inoculation.

Initiation of culture:

The auxiliary bud (AXBG) and apical bud (APBG) explants were initiated (Fig. 2.0) on MS media [12] supplemented with 1mg/l BAP (6- Benzyl amino purine) having 0.6% Agar and 3% Sucrose as carbon source. pH of the medium was adjusted to 5.8. The initial cultures were incubated at 25 ± 2 °C with 16 hr photoperiod (using cool fluorescence light 50 μ Em⁻² Philip India) for 4 weeks. The auxiliary bud was observed to have better response as explants in terms of shoot initiation as compare to apical bud.

Multiplication

After 4 weeks of incubation of 1st stage cultures, the new initiated shoots were dissected out and transferred on MS media (Fig. 3.0 and 4.0) with various concentrations of BAP (Table 1.1). Each treatment had 10 multiplications and the experiment was repeated twice. Ten random culture bottles from each accession were taken for observing various morphological characters like shoot length and number of multiple shoots. Though initial response was observed in apical bud explants but it was not found to be appropriate for further sub culturing.

Table: 1. Different medium used for shoot multiplication.

S. No.	Acc. No.	No. of bottles	Media Strength	Growth Hormone in mg/L
1	ABG 1	10	MS	Hormone free
2	ABG 2	10	MS	1.0 BAP
3	ABG 3	10	MS	2.0 BAP
4	ABG 4	10	MS	4.0 BAP
5	ABG 5	10	½ MS	Hormone free
6	ABG 6	10	½ MS	0.5 BAP
7	ABG 7	10	½ MS	1.0 BAP
8	ABG 8	10	¾ MS	Hormone free
9	ABG 9	10	¾ MS	0.5 BAP
10	ABG 10	10	¾ MS	1.0 BAP

Rooting

After 3rd subculture stage, elongated shoots having multiple leaves were excised from the multiple shoots clump. These shoots were transferred into the rooting media supplemented with different concentration of IBA (Indole butyric Acid) (Table 1.2). *in vitro* root inductions of well established shoots were observed (Fig. 5.0) as mentioned in Graph 3.0. The following media compositions were used for the *in vitro* induction of roots for bitter gourd.

Table: 2. Different media composition used for root induction.

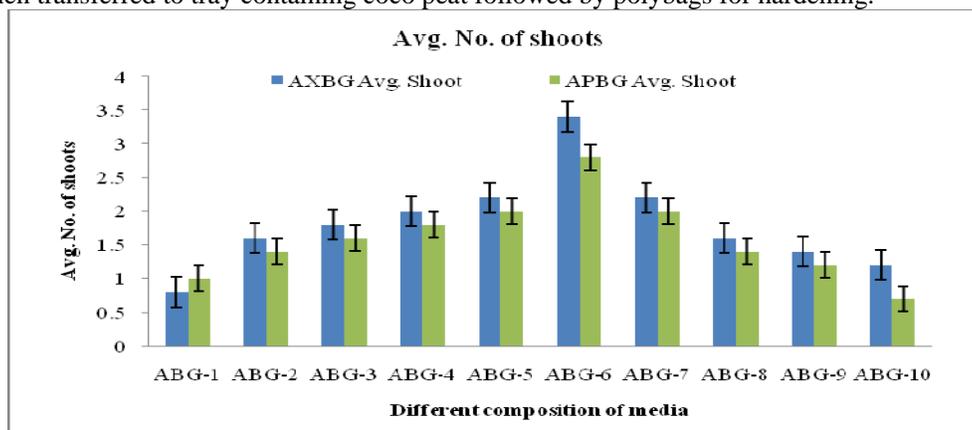
S. No.	Acc. No.	No. of bottles	Media Strength	Growth Hormone in mg/L
1	RBG 1	10	MS	Hormone free
2	RBG 2	10	MS	MS + 0.5 IBA
3	RBG 3	10	MS	MS + 1.0 IBA
4	RBG 4	10	MS	MS + 2.0 IBA
5	RBG 5	10	½ MS	Hormone free
6	RBG 6	10	½ MS	MS + 0.5 IBA
7	RBG 7	10	½ MS	MS + 1.0 IBA
8	RBG 8	10	½ MS	MS + 2.0 IBA

Hardening

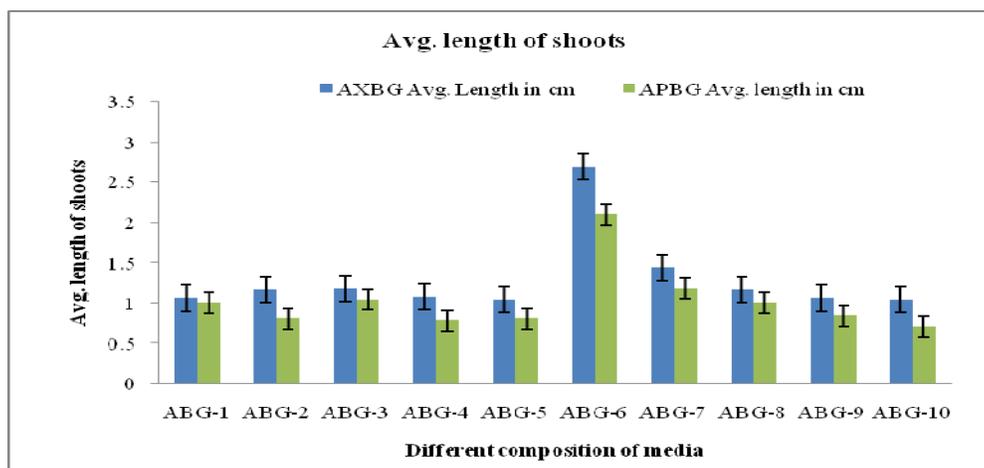
The rooted plantlets were withdrawn from the media and were thoroughly washed with running tap water to remove adhering media followed by treatment with systemic fungicide (Bavistin-0.1%). For primary hardening the ex-agar plantlets were transferred to green house and planted in trays containing coco peat. The primary hardened plants were transferred to shade house and planted in polythene bags filled with FYM+ Soil+ Sand (3:2:1) for a period of 35-40 days for secondary hardening (Fig.6.0).

RESULT AND DISCUSSION

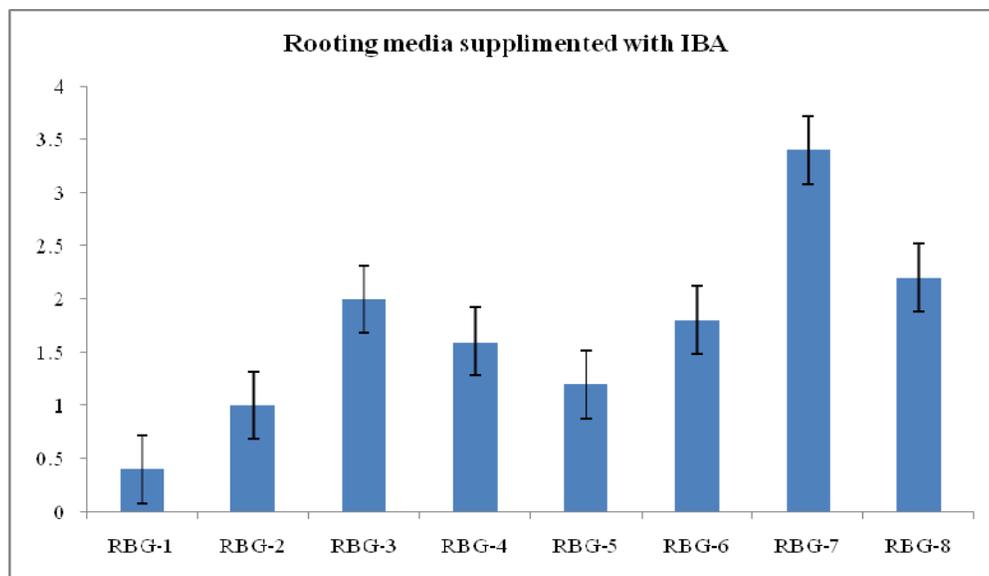
The present study was carried out to optimize shooting and rooting media for *in vitro* propagation of gynocious lines of bitter melon. The effect of MS medium supplemented with growth regulators on shoot induction in Axial (AXBG) and Apical bud (APBG) is summarised in Graph. 1.0. And 2.0 respectively. Regeneration was observed from both types of explants. The auxiliary bud as explants was better than that of apical bud in terms of bud breaking response. Similar results had been also reported by Mala Agrwal *et al.*, (2004) [1]. Regeneration of small shoots started appearing within 9-11 days in all culture bottles. After 30 days of incubation maximum average number of shoots were obtained in ABG-6 medium which were recorded to have 3.4 shoots in AXBG and 2.8 shoots in APBG (i.e. ½ MS media supplemented with 0.5 mg/ml BAP) respectively (graph 1.0). The average shoot length recorded in ABG-6 medium was found to be 2.7cm in AXBG and 2.1 cm in APBG respectively (graph 2.0), whereas minimum average shoot length of 1.04 cm in (AXBG) ABG-5 and 10 medium and 0.7 cm in (APBG) ABG-10 medium respectively was observed. The Different concentration of BAP in shooting medium was found to be effective for multiplication of the shoots. The shoots in ABG-6 medium were active and healthy compare to other media. It was also observe that shoots in APBG were as elongated as in AXBG but the shoots were found succulent and weak. The regenerated shoots of AXBG were further used for the root induction in root forming media. The average root formation response was different in all media. The highest average number of roots formed was 3.4 in RBG-7 medium (Graph 3.0) similar results were observed when Minocha *et al.*, 1987 [10] used same rooting media as we used in this study. The least average root formation was recorded 0.4 in RBG-1 media (Graph 3.0). Root formation took almost 15 days for initiation on rooting media. It was observed that IBA alone was effective in the induction of roots in bitter melon [1]. The similar results were also reported in other plants like *Limonium altaica*, L. by Jeong *et al.*, (2001) [6]. Further the cultured plantlets were withdrawn from the rooting media and then transferred to tray containing coco peat followed by polybags for hardening.



Graph 1.0: Average number of shoots after 3rd subculture stage of auxiliary bud (AXBG) and apical bud (APBG) explants.



Graph 2.0: Average length of shoots after 3rd subculture stage of auxiliary bud (AXBG) and apical bud (APBG) explants.



Graph 3.0: Average number of roots observed in different media used for root induction.



Fig. 1.0 Mother plant of bitter gourd



Fig.2.0: Initiation stage of apical bud explants.



Fig.3.0: Multiplication stage of apical bud



Fig.4.0: Multiplication stage of axial bud



Fig.5.0: Rooting stage of axial bud explants.



Fig.6.0: Hardening stage.

CONCLUSION

In this study it was found that the small shoots started appearing within 9-11 days in all culture bottles. Auxiliary bud explants were better in all stages involved in micro propagation starting from initiation to hardening. After 30 days of incubation maximum shoot length and number of shoot were obtained in ABG-6 media (MS supplemented with 0.5 mg/ml BAP). A highest average root formation was observed in RBG-7 followed by RBG-8 medium. Although some progress has been made in the regeneration of gynoecious bitter gourd but there is much work need to be done in this direction. We believe that this study will help in establishment of an efficient and reproducible regeneration of gynoecious lines of bitter gourd through basic tissue culture technique, and will be beneficial for most biotechnology mediated crop improvement and hybrid production program.

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