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Development of *invitro* Regeneration Protocol in Bitter Gourd (*Momordica charantia*)

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A B S T R A C T

In the present study an attempt was made for *in vitro* regeneration of bitter gourd cv. "BSS-1007". In plant breeding tissue culture techniques play as an important role for rapid multiplication of particularly desirable genotype of F_1 hybrids, gynoecious lines, triploid hybrid plants *etc*. where seed costs are high. Seeds of bitter gourd cv. BSS-1007 were surface sterilized with bavistin (0.1%) for 1 hr. followed by 70% ethanol (v/v) for 1 minutes and then 0.1% $HgCl_2$ (w/v) solution for 5 minutes. After sterilization seeds were cultured on ½ MS medium for explants preparation. For direct multiple shoot induction, auxiliary bud and shoot apical meristem explants were cultured separately on MS media supplemented with different concentrations of BAP. Among these combinations, 1.0 mg/l BAP was found most effective for shoot induction from auxiliary buds producing maximum 3.19 numbers of shoots with minimum 7.30 days required for shoot initiation. While, MS medium fortified with 1.5 mg/l BAP was suitable for shoot induction from shoot apical meristem producing maximum 2.33 numbers of shoots with minimum 7.67 days required for shoot initiation. *In vitro* rooting of shoots was done by using MS medium fortified with different concentrations of IBA. Among these different rooting media, MS medium supplemented with 2.0 mg/l IBA was best for rooting on which highest average number of roots (40.71) were produced with minimum average days required for root induction (6.57days). Rooted plantlets were washed thoroughly and transferred to polythene cups containing coco-peat and acclimatized in green house condition.

Introduction

Bitter gourd; also known as balsam pear, bitter melon or bitter cucumber is an important vegetable crop belonging to family *Cucurbitaceae*, is a dicotyledonous seasonal vegetable having somatic chromosome number 2n=22. Bitter gourd is cultivated throughout the entire tropics and subtropics (Saha and Behera, 2015). This is one of the important vegetable in our diet since ancient time due to its important nutritional and medicinal values. Fruits of bitter gourd have great Medicinal properties include anti-microbial, anti-mutagenic, anticancerous, anti-infertility and antidiabetic properties (Singh *et al.*, 1998), are also rich source of vitamin A, vitamin C, thymine,

riboflavin and mineral hence it is considered as most nutritive vegetable among all cucurbits.

Conventional breeding practices for crop improvements are time-consuming. Applying modern biotechnology techniques are important for the crop improvement in bitter gourd (Sinha *et al.,* 2019). Plant tissue cultures have wide applications in plant breeding, commercial production and basic biological research (Agarwal, 2015).

In crop improvement tissue culture play as an important role for rapid multiplication of particularly desirable genotype of F_1 hybrids, gynoecious lines, triploid hybrid plants *etc*. where seed costs are high. 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 alternative approach for multiplying gynoecious lines of bitter gourd through *in vitro* micropropgation will be of great importance in hybrid seed production at commercial level (Saha and Behera, 2015).

With this aim, the medicinally important bitter gourd has been taken for the present investigation. The present work was undertaken to study the response of bitter gourd to the *in vitro* regeneration using optimum concentration of plant growth regulators with objective to develop a reliable micropropagation protocol in bitter gourd.

Materials and Methods

Genotype

In the present study, the commercial hybrid cultivar of bitter gourd cv. BSS-1007 was used. Seeds of bitter gourd cv. BSS-1007 (Figure 1a) were collected from Kalash Seeds Private Limited, Jalna (Maharashtra, India).

Seed sterilization

The seeds of bitter gourd 'BSS-1007' were surface sterilized with 0.1% bavistin for 1hr followed by 3 times washing with sterilized autoclaved water. Then seeds were treated with 70% ethanol (v/v) for 1 min followed by washing twice with autoclaved water. Lastly seeds were treated with 0.1% $HgCl_2$ (w/v) solution for 5 minutes followed by thorough washing for 5 times with autoclaved water (Figure 1b).

Preparation of explants

The sterilized seeds of BSS-1007 were germinated on ½ MS medium under *in vitro* conditions (Figure c). The shoot apical meristem and auxiliary bud explants were excised from 10-12 days old *in vitro* grown seedlings of bitter gourd and used for shoot induction. The culture bottles were kept in dark room at 25±2^{0}C temperature till germination. After germination culture bottles were transferred into light room maintained at 25±2°C and 1600 lux light intensity.

Shoot elongation from apical meristem and auxillary bud

The explants were prepared for inoculation by cutting shoot apical meristem and auxiliary bud portion in proper size and removing all leafs under aseptic condition. The explants were then inoculated aseptically in sterile culture bottles containing MS (Murashige and Skoog, 1962) media along with different concentrations of BAP (0.0, 0.5, 1.0, 1.5 and 2.0 mg/l) for shoots elongation. Then culture bottles were incubated in culture room maintained at 25±2°C and a photoperiod of 16/8 hours at 1600 lux light intensity. Cultures were observed regularly for microbial contamination or browning of explants.

Invitro root formation

The explants were prepared for inoculation by separating the multiple shoot and removing debris under aseptic condition. The explants were then inoculated aseptically on sterile culture bottles containing MS media fortified with different concentrations of IBA (0, 0.5, 1.0, 1.5 and 2.0 mg/l).

Hardening

Rooted plantlets were subjected to stepwise acclimatization and hardening. Well rooted plantlets after removing agar were first treated thoroughly with bavistin. The healthy plants were transferred to plastic cups containing coco-peat and kept in polyhouse for primary hardening. After the primary hardening plants

were transferred to the potting mixture containing coco peat mixture and kept in greenhouse condition for secondary hardening.

Results and Discussion

Multiple shoot induction from auxiliary bud explants

Effect of four different concentrations of BAP along with control (MS media without BAP) on multiple shoot induction was studied in bitter gourd. The auxiliary buds derived from 10-12 days old *in vitro* grown seedlings were used as explants for direct shoot induction. These explants were cultured on MS medium supplemented with different concentrations of BAP (0.0, 0.5, 1.0, 1.5 and 2.0 mg/l) for shoot induction.

Among these different media, MS medium fortified with 1.0 mg/l BAP was found suitable for multiple shoot induction from auxiliary bud explants which produces 3.19 average number of shoots followed by 2.07 shoots on MS medium with 0.5 mg/l BAP (Figure 2a). While lowest average numbers of shoots were observed on MS medium without BAP (Table 1). MS medium supplemented with 1.0 mg/l BAP was found best for average number of days (7.30 days) required for shoot initiation, followed by MS medium containing 2.0 mg/l BAP requires 7.40 days. Maximum days were required for shoot induction in MS medium without any growth regulator (Table 1).

Multiple shoot induction from shoot apical meristem explants

As like auxiliary bud explants, effect of four different concentrations of BAP along with control i.e. MS media without BAP on multiple shoot induction from shoot apical meristem explants was also studied in bitter gourd. Shoot apical meristem explants derived from 10-12 days old *in vitro* grown seedlings were cultured on MS medium supplemented with different concentrations of BAP (0.0, 0.5, 1.0, 1.5 and 2.0 mg/l) for multiple shoot induction.

Among the different combinations used for multiple shoot induction from shoot apical meristem explants, MS medium supplemented with 1.5 mg/l BAP was found suitable which produces 2.33 average number of shoots followed by 1.83 shoots on MS medium with 2.0 mg/l BAP (Figure 2b). Whereas lowest average numbers of shoots were observed on MS medium without BAP (Table 2). Minimum average number of days for shoot initiation was found to be 7.67 days on MS medium supplemented with 1.5 mg/l and 2.0 mg/l BAP followed by 10 days on MS medium containing 1.0 mg/l BAP (Table 2).

Shoot elongation

The multiplied shoots are further separated from the clumps and transferred in to the MS medium without any growth regulators for elongation. After the two weeks of growth, shoots was elongated well with sufficient number of roots (Figure 2c and 2d). The rooted plantlets are hardened separately, and found less percent of survival. This is may be due to the less number of roots or the fragile nature of roots formed during elongation.

Rooting

The regenerated shoots were transferred to MS medium supplemented with different concentrations of IBA (0.0, 0.5, 1.0, 1.5 and 2.0 mg/l) for rooting and the root characteristics were observed viz. number of roots, root length and number of days for root initiation.

Minimum average days required for root induction was found to be 6.57 on MS medium supplemented with 2.0 mg/l IBA followed by 7.33 days on MS supplemented with 1.5 mg/l IBA, while root induction was late in medium without IBA (Table 3).

The highest average number of roots (40.71) were obtained on MS medium fortified with 2.0 mg/l IBA followed by 33.24 roots on MS medium supplemented with 1.5 mg/l IBA. However lowest number of roots (3.90) were produced on MS medium without growth hormone (Table 3).

In case of root length, maximum average root length (2.62 cm) was observed on MS medium without IBA followed by 2.0 cm on MS medium supplemented with 0.5 mg/l IBA. The minimum root length (1.04 cm) was observed on MS medium with 2.0 mg/l IBA (Table 3). It was observed that, as the concentration of IBA increases, the number of roots increases, while length of root decreases. The effects of different concentrations of IBA on root formation in bitter gourd cv. BS-1007 were presented in figure 3.

Table.1 Effect of BAP on in vitro shoot induction of bitter gourd from auxiliary bud explants

Sr. No.	Media composition	Average No. of shoots /explant (Mean ± SE)	Average No. of days for shoot initiation (Mean± SE)
1	MS+BAP (0.0 mg/l)	1 ± 0.00	11.30 ± 0.45
2	MS+BAP (0.5 mg/l)	2.07 ± 0.08	8.40 ± 0.34
3	MS+BAP (1.0 mg/l)	3.19 ± 0.05	7.30 ± 0.30
4	MS+BAP (1.5 mg/l)	1.81 ± 0.08	7.70 ± 0.30
5	MS+BAP (2.0 mg/l)	1.67 ± 0.09	7.40 ± 0.27

Table.2 Effect of BAP on *in vitro* shoot induction of bitter gourd from shoot apical meristem

Sr. No.	Media composition	Average No. of shoots/explants (Mean ± SE)	Average No. of days for shoot initiation (Mean ± SE)
1	MS+BAP (0.0 mg/l)	1.00 ± 0.01	11.50 ± 0.43
2	MS+BAP (0.5 mg/l)	1.78 ± 0.05	10.50 ± 0.56
3	MS+BAP (1.0 mg/l)	1.83 ± 0.04	9.83 ± 0.40
4	MS+BAP (1.5 mg/l)	2.33 ± 0.07	7.67 ± 0.33
5	MS+BAP (2.0 mg/l)	1.83 ± 0.04	8.00 ± 0.52

Table.3 Effect of IBA on root formation in bitter gourd

Sr. No.	Media composition	Average No. of days for Root initiation (Mean ± SE)	Average No. of Roots (Mean ± SE)	Average Root length (cm) (Mean ± SE)
1.	MS+IBA (0.0mg/l)	8.95 ± 0.40	3.90 ± 0.24	2.62 ± 0.06
2.	MS+IBA (0.5mg/l)	8.29 ± 0.38	13.43 ± 0.56	2.00 ± 0.06
3.	MS+IBA (1.0mg/l)	7.52 ± 0.41	24.43 ± 0.94	1.73 ± 0.06
4.	MS+IBA (1.5mg/l)	7.33 ± 0.26	33.24 ± 0.80	1.40 ± 0.00
5.	MS+IBA (2.0mg/l)	6.57 ± 0.28	40.71 ± 0.77	1.04 ± 0.07

Figure.1 Explant preparation from seedling culture of bitter gourd cv. BSS-1007 (a: bitter gourd seeds, b: sterilization of seed, c: seedling culture)

Figure.2 Multiplication and shoot elongation (a: multiple shoot induction from auxiliary bud explants, b: multiple shoot induction from apical meristem shoot tip, c: Shoot elongation, d: roots formed during shoot elongation)

a b c d

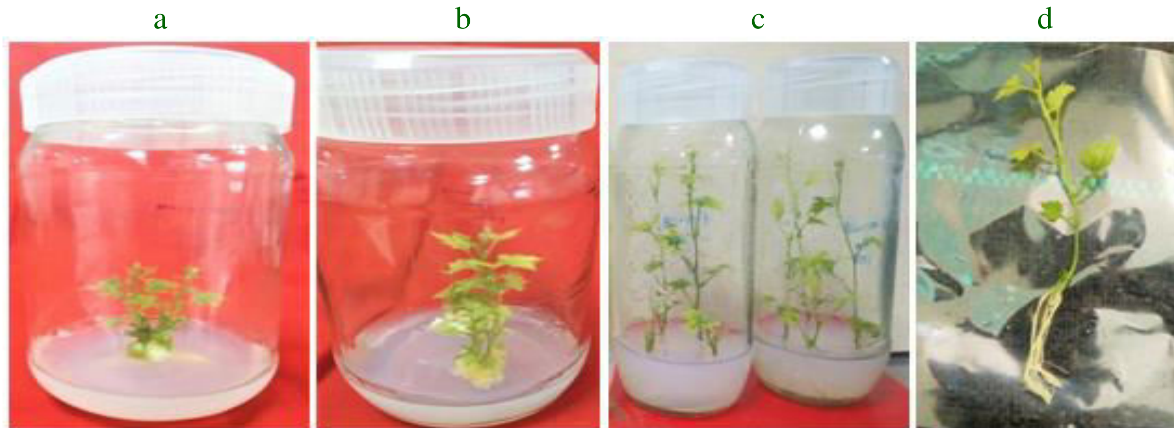

Figure.3 Effect of different concentration of IBA on root induction (a: 0.0 mg/L, b: 0.5 mg/L, c: 1.0 mg/L, d: 1.5 mg/L, e: 2.0 mg/L)

Figure.4 Hardened plants of bitter gourd

Hardening

After two weeks of primary hardening in polyhouse and three weeks of secondary hardening in greenhouse conditions the 89 % plants of in vitro regenerated bitter gourd cv. BSS-1007 were survived healthy successfully. The plants of bitter gourd cv. BS-1007 after two weeks of secondary hardening in greenhouse conditions were presented in figure 4. An *in vitro* regeneration study in bitter gourd cv. “BSS-1007” *was* done using shoot apical meristem and auxiliary bud explants. For direct multiple shoot regeneration, Shoot Apical meristem and Auxiliary bud explants derived from 10-12 days old *in vitro* grown seedlings were cultured on different concentrations of BAP (0.0, 0.5, 1.0, 1.5, and 2.0 mg/l). Among these different media, MS medium supplemented with 1.0 mg/l BAP was most suitable for multiple shoot induction from auxiliary buds with maximum 3.19 average shoots. While for Shoot Apical meristem explants, MS medium fortified with 1.5mg/l BAP was best with maximum of 2.33 average numbers of shoots. These findings are similar with Huda and Sikdar (2006) who had reported the micro propagation of bitter gourd using shoot apical meristem on Murashige and Skoog medium, supplemented with 1 mg/l BAP. Agarwal and Kamal (2004) and Verma *et al.,* (2014) also reported that 0.5 mg/l BAP is best for multiplication of *in vitro* germinated auxiliary bud explants of bitter gourd. For *in vitro*

germinated apical meristem of bitter gourd 2.0 mg/l BAP was found to be best for shoot induction (Agarwal and Kamal, 2004). Use of BAP has given best response for shoot induction in bitter gourd than other harmones was reported by several authors such as Sultana *et al.,* (2005); Munsur *et al.,* (2007) and Munsur *et al.,* (2009).

The shoot elongation is necessary for growth of the shoots with increased strength and height. In the present study multiplied shoots obtained from both type of explants are separated from the clumps and transferred in to the elongation medium for two weeks. Well elongated shoots with developed roots was hardened separately to analyze acclimatization performance and found less percent of survival. Due to elongated shoots are failed at the acclimatization stage the new multiplied shoots are rooted on MS medium supplemented with different concentrations of IBA. Among these different medium, the highest number of roots (40.71) was obtained on MS medium fortified with 2.0 mg/l IBA with minimum average days required for root induction (6.57 days). Maximum root length (2.62) was found on MS media without hormone. It was also observed that as the concentration of IBA increases the root number increases and root length decreases. Similar results were also reported by Ugandha *et al.,* (2014) who obtained maximum rooting frequency on MS medium supplemented with 1.5 mg/l IBA. Use of IBA for root induction in bitter gourd with high percent of rooting was reported by Verma *et al.,* (2014). The *in vitro* raised plantlets were successfully established in green house conditions with 89 % survival rate.

Author Contribution

Anil N. Kale: Investigation, formal analysis, writing—original draft. Pradip M. Adlinge: Validation, methodology, writing—reviewing. Vaibhav G. Waghmare:—Formal analysis, writing—review and editing. Vijay K. Raut: Investigation, writing—reviewing. Sachin L. Abhang: Resources, investigation writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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