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Research Article

Exploiting shoot tips as an efficient explant for *in vitro* regeneration of cucumber (*Cucumis sativus* L.)

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Abstract

A proficient and speedy *in vitro* regeneration is a prerequisite for genetic modification of plants. The described protocol presents an efficient and practicable procedure for regeneration and multiple shoots induction by using shoot tips as explants for cucumber (*Cucumis sativus* L.). First, aseptic seedlings were established and shoot tips were excised from those seedlings. The cucumber seedlings used after 7 days of germination were found to be the best source of explants. Theses shoot tips were placed on MS medium supplemented with different concentrations of Benzyl amino purine (BAP) (0.5-2.5mg/L). It was observed that MS medium augmented with 1.5mg/L BAP resulted in maximum shoots formation. By increasing BAP beyond 1.5mg/L, rate of shoots formation was decreased. For rooting purpose, Indole Butyric Acid (IBA) (0.5-2.5mg/L) was used. Shoots greater than 2cm in size were shifted to rooting medium. Best roots development took place on MS medium having 1mg/L IBA. The plantlets with well-developed roots were shifted to peat moss containing pots and gradually acclimated to soil. This *in vitro* regeneration protocol can be used for genetic transformation of cucumber in future.

Keywords: BAP; Cucumber; IBA; *In vitro* regeneration; Shoot tips

Introduction

Cucumber (*Cucumis sativus* L.), is a member of family *Cucurbitaceae* and genus *Cucumis*. This genus is comprised of 52 species **[1]**. Cucumbers and melons are two most economically important food crops. Cucumber has gained much attention to be used as model plant for research purpose in family *Cucurbitaceae* because its genome sequence is available and efforts have been made to transform its genome **[2]**. It is a monoecious vegetable with vining type stem which is grown worldwide **[3]** and being used in several bouquet perfumes. Cucumber has tonic, refrigerant and diuretic seeds **[4]** which have many applications in cosmetics and medicine. Strong antioxidant potential of cucumbers makes them suitable to irritated skin caused by sun burn for its soothing and chilling effect **[5]**.

Cucumber is a vegetable crop of great importance, susceptible to various viral and bacterial diseases which limit its average yield. Moreover, cucumber is vulnerable to

several fungal pathogens especially when cultivated in tunnels where semi-closed environment with elevated humidity and temperature regimes maximize the chances of fungal infestation. Hence, production of cucumber lines resistant to viral, bacterial and fungal diseases is desirable in all Cucumber growing areas around the globe.

Cucumber has narrow genetic base makes the use of classical breeding a bit tedious for cucumber varietal improvement. Crossing incompatibility is also a barrier in undertaking inter-specific hybridization of cucumber with other members of the genus *Cucumis* and family *Cucurbitaceae*. Hence, the development of somaclonal variants, somatic hybrids and transgenic lines are some practical options available for improvement of cucumber crop **[6, 7]**. All these methodologies need highly effective tissue culture regeneration system for crop improvement **[8, 9]**. As modern biotechnological techniques including gene transfer technologies are being used for crop improvement **[10]**. The aim of present research was to standardize the concentrations of the plant growth regulators (PGRs) for improving *in vitro* regeneration of cucumber which can be used to produce transgenic cucumber lines in future through gene transfer technologies.

Materials and methods

In vitro establishment of aseptic seedlings

Seeds of cucumber variety namely, "Desi" were obtained from horticulture department of University of Agriculture, Faisalabad. First, healthy seeds were washed 3-4 times with double distilled water. Then, seeds were treated with 70% ethanol for 2 minutes, 0.2% mercuric chloride having 2 drops of tween-20 for 5 minutes. Finally, seeds were washed with sterile distilled water 5-6 times until all the mercuric chloride was removed. Then, seeds were de-coated using autoclaved forceps and placed on MS medium (Murashige and Skoog) without any growth regulators and incubated in dark at 25±2 °C for the *in vitro* establishment of seedlings.

Culture medium and conditions

The media employed in the current study contained Murashige and Skoog (MS) salts **[11]** which were then supplemented with different concentrations and combinations of PGRs namely 6-benzylaminopurine (BAP) (0.5-2.5mg/L), zeatin BAP (2.0mg/L), and indole-3-butyric acid (IBA) (0.5-2.5mg/L) as given in (Table 1). At the end, pH was adjusted to 5.8 before autoclaving.

Explant attainment

In vitro grown seedlings of cucumber after 7, 8 and 9 days of culture were used to obtain shoot tips. Shoot tips were placed on MS media having 0.5-2.5 mg/L BAP and 2mg/L zeatin. By using shoot tips as explants, direct shoots induction was observed by providing 25±2 °C under 16 hours light and 8 hours dark conditions.

Table 1. Exact concentrations of different plant growth regulators (PGRs) used in this study

Sr. No.	Shooting media Conc. (mg/L)	Rooting media Conc. (mg/L)
1	BAP (0.5) Zeatin (2.0)	IBA (0.5)
2	BAP (0.1) Zeatin (2.0)	IBA (1.0)
3	BAP (1.5) Zeatin (2.0)	IBA (1.5)
4	BAP (2.0) Zeatin (2.0)	IBA (2.0)
5	BAP (2.5) Zeatin (2.0)	IBA (2.5)

Rooting and acclimatization

Regenerated shoots greater than 2cm in length were shifted to rooting medium having different concentrations of IBA. Plants were placed on the rooting medium for 2-3weeks till vigorous rooting. Firstly, roots were washed with tap water. Well-developed rooted plants were shifted to plastic pots having autoclaved peatmoss. Polythene bags were used to cover the pots to minimize evapo-transpirational losses. Gradually, bags were removed after some weeks and plants were shifted to green house for acclimatization.

Results

In vitro growing seedlings were used as source of explants. The 7, 8- and 9-days old seedlings were used for this purpose. The age of seedlings was found to be an important factor in the determination of regeneration rate. The 7 days old seedlings responded well and maximum for regeneration. By increased ages of seedlings more than 7 days, rate of regeneration was decreased. Moreover, shoot tips proved to be a reasonable explant for *in vitro* regeneration of cucumber.

Multiple shoots regeneration

Shoot tips originating in between the cotyledons were carefully excised from *in vitro* growing seedlings. When shoots tips were placed on regeneration medium having 2mg/L zeatin, it resulted in the development of direct shoots regeneration. But rate of shoots development was very slow. It was observed that multiple shoots induction took place by using MS medium augmented with different concentrations of BAP (0.5-2.5mg/L) and 2mg/L zeatin (Table 1). By using different concentrations of BAP, rate of shoots formation was different. However, response of shoots induction on medium having 0.5mg/L BAP was very slow which increased with the increase in BAP concentration. It was recorded that MS medium augmented with 1.5mg/L BAP produced maximum number of shoots (Figure 1) as compared to other concentrations of BAP being used. The number of shoot regeneration decreased by using concentrations above 1.5mg/L BAP. Concentration of BAP being used was an important factor in determination of shoots regeneration rate.

Roots induction

For rooting purpose, elongated shoots greater than 2cm in length were cultured on MS medium having different concentrations of IBA (0.5-2.5mg/L) (Table 1). It was observed that adventitious roots emerged from base of the shoots directly. By using different concentrations of IBA, variable number of roots was observed however, maximum roots were obtained by using 1.0mg/L IBA. Rate of roots formation increased up to 1.0mg/L concentration and beyond this level, number of roots regeneration was decreased.

Discussion

For the purpose of *in vitro* direct shoots formation in *Cucurbitaceae*, BAP and zeatin are found to be effective. By using shoot tips of cucumber as explants, maximum number of shoots were produced on medium having 1.0mg/L BAP **[12]**. Lee *et al*. **[13]** reported shoot bud regeneration in winter squash (*Cucurbita maxima*) using MS medium supplemented with BAP. The BAP was found to be the most important plant growth regulator for shoots formation in squash (*Cucurbita pepo*) by using 1.0mg/L BAP concentration **[14]**. Similarly, Agarwal and Kamal **[15]** reported that use of BAP was necessary for shoot formation in bitter gourd (*Momordica charantia*). Similar, results were found in bottle guard (*Lagenaria siceraria*) where BAP 2.0mg/L played an important role in enhancement of shoots regeneration **[16]**. Later, induction of multiple shoot buds from cotyledonary explants was observed in watermelon (*Citrullus lanatus*) using BAP **[17]**. The results reported in the present study are in line with the above cited literature and BAP 1.5mg/L has been found effective for

inducing multiple shoots from shoot tips (Figure 1).

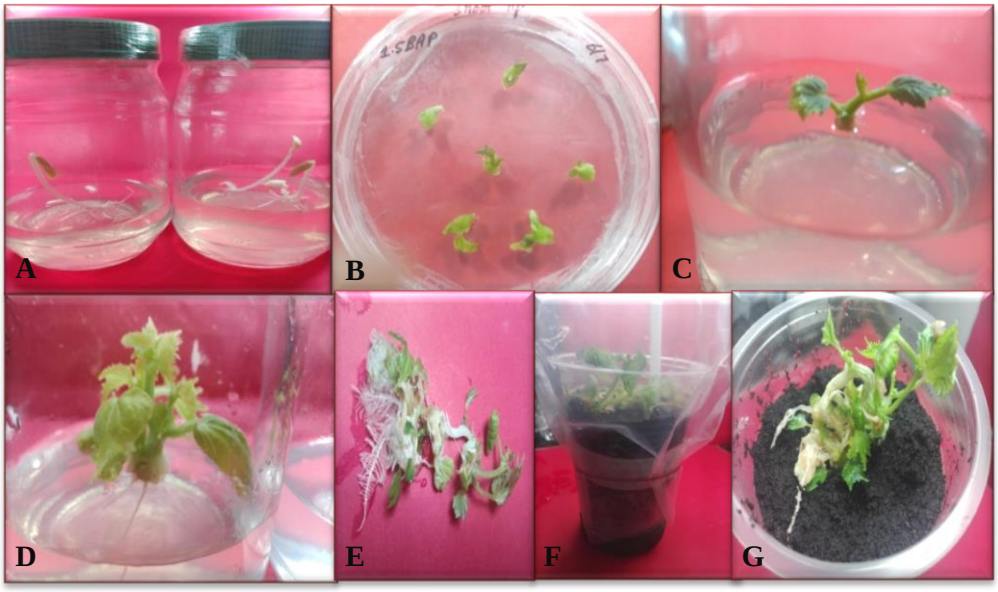

Figure 1. Stepwise *in vitro* regeneration of cucumber. (A) Seven days old seedlings grown from surface sterilized de-coated seeds on MSO medium (B) Excised shoot tips placed on SIM (C) Shoots growing from shoot tips on SIM (D) Shoots multiplication on SIM (E) Roots development on medium having IBA (F) Plantlets shifted into peat moss and covered with polythene bags (G) Plants growing in peat moss after removing polythene bags

Indole Butyric Acid (IBA) has been found effective in root induction. Selvaraj *et al*. [18] reported that IBA at concentration of 0.7mg/L was effective in roots induction for cucumber. It is reported that IBA plays an important role in roots induction for the other members of the family *Cucurbitaceae* like teasle gourd and winter melon [19, 20]. Similarly, Rai *et al*. [21] used IBA for rooting purpose in *M. dioica*. Some authors have reported zeatin to encourage adventitious shoot formation in cucumber at a low concentration as described in present study [22-24].

Conclusion

The cucumber (*Cucumis sativus* L.) being vulnerable to various biotic stresses needs to be improved using modern biotechnological tools including genetic transformation which requires efficient tissue culture system in most cases. The reported method employed shoot tips as proficient explants for regeneration of multiple shoots when cultured on MS medium supplemented with 1.5mg/L BAP and 2mg/L zeatin. This protocol may help in genetic transformation of cucumber.

Authors' contributions

Conceived and designed the experiments: FA Joyia & MS Khan, Performed the experiments: R Zahra & MN Anjum, Analyzed the data: FA Joyia & S Munawar, Contributed reagents/ materials/ analysis tools/ facilities: G Mustafa & MS Khan, Wrote the paper: FA Joyia, G Mustafa & R Zahra.

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