The influence of plant growth regulators on callus induction from hypocotyls of cucumber (Cucumis sativus L.)

Saeid Abu-Romman, Mohammad Suwwan and Ezz AL-Dein Al-Ramamneh

Department of Biotechnology, Al-Balqa Applied University, Al-Salt 19117, Jordan.
Department of Horticulture and Crop Science, University of Jordan, Amman, Jordan
Department of Agricultural Sciences, Al-Shouback University College, Al-Balqa Applied University, Al-Shouback, Maan, Jordan

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ABSTRACT

The purpose of this study was to develop an efficient protocol for callus induction of cucumber. Hypocotyl explants of cucumber (Cucumis sativus L. cv. Sultan) were cultured on Murashige and Skoog (MS) media supplemented with individual treatments of different auxins (2,4-dichloro-phenoxyacetic acid (2,4-D), indole-3-acetic acid (IAA), α-naphthalene acetic acid (NAA)) or cytokinins (benzyl adenine (BA) and kinetin (Kn)). The result of this study showed that callus induction frequency, callus growth rate and nature of callus were significantly affected by type and concentration of the plant growth regulators and were greatly higher when incorporating auxins in the medium compared to cytokinins. The maximum callus induction frequencies was observed on MS media with 2,4-D or NAA. NAA at 1.0 mg/L resulted in the best callus growth index. Incorporating cytokinins (BA or Kn) in the callus induction media promoted slow callus growth and low quality callus compared to that produced on media containing auxins. Callus induced on media containing NAA or IAA was friable and yellow in color.

Key words: Callus induction; cucumber; plant growth regulators

Introduction

The Cucurbitaceae is one of the most important families worldwide as sources of vegetables. There are about 118 genera and 825 species of annual vines recognized in the Cucurbitaceae [1]. Cucurbit is becoming continuously used as a general term for all taxa in the family [2]. The four major economically important crops of the cucurbits are watermelon (Citrullus lanatus (Thunb.), cucumber (Cucumis sativus L.), melon (Cucumis melo L.) and squash (Cucurbita spp.) [2]. The genus Cucumis contains 52 species, of which cucumber and melon are the two most economically important food crops [3]. The cucumber is thought to have been first domesticated in central Asia [4]. Botanically, cucumber is a monoecious, annual, herbaceous and vining plant. Cultivated cucumbers are distributed throughout the world and are the fourth most important vegetable crop behind tomato, cabbage and onion [5].

Conventional breeding programs and transfer of desirable traits, especially stress resistance from wild species have not been successful in Cucumis [6]. The intention of modern biotechnology is the improvement of crops by means that are impossible by classical plant breeding [7]. In order to achieve the goal of modern biotechnology for the development of transgenic cucumber plant, successful and efficient in vitro regeneration system is required [8]. The use of molecular biotechnology in cucurbits genetic improvement solved many problems associated with traditional breeding techniques [9,10].

In vitro regeneration of cucumber is possible using various culture techniques [11]. A variety of explants have been used for plant regeneration from cucumber via organogenesis viz., cotyledons [12,13,14], primary leaves [11,15,16,17,18], petioles [19] and hypocotyls [14,20,21,22]. Regeneration of cucumber is also possible through suspension culture [15,23,24,25] or by somatic embryogenesis in solid medium [14]. Cucumber in vitro culture has been recently used to evaluate cucumber physiological responses to osmotic stress [26,27]. Several protocols for the genetic transformation of cucumber [28,29,30,31,32] have been established utilizing indirect organogenesis procedures.

Despite the availability of various publications on cucumber organogenesis and genetic transformation, a careful perusal reveals that the
frequency of regeneration/transformation was comparatively low and it was dependent to a greater extent on the type of the explants, the cultivars, hormone combinations and physical conditions of culture. Wehner and Locy [33] and Kim et al. [13] already indicated that the success of cucumber regeneration was largely genotype dependent. Therefore, the aforesaid facts constantly call for improved regeneration protocols for cucumber genotypes for their genetic improvement. Accordingly, the present work was undertaken to establish an efficient and reproducible protocol for callus induction from hypocotyl explants of commercially important slicing cucumber cv. Sultan.

Materials and Methods

Seeds of cucumber cv. Sultan were procured from Petoseeds, (CA. USA). Seeds were decaoted after soaking in distilled water for 15 minutes. The decaoted seeds were surface sterilized by soaking in 70% ethanol for 1 minute, then immersed for 25 minutes in 1% sodium hypochlorite with 2 drops of Tween 20 per 100 ml. Finally the seeds were rinsed three times with sterile distilled water.

The sterilized decaoted seeds were germinated on petri dishes containing modified MS [34] bioregulators free medium supplemented with 1% agar (Bacto) and 3% sucrose (Sigma). The culture was maintained at 22 °C for 6 days under 16 hr photoperiod with the light intensity of 40 μmol m⁻² s⁻¹.

For callus induction, the basal part of the 6 day old seedling hypocotyls were dissected (0.5 mm) and inoculated on callus induction Murashige and Skoog’s medium [34] containing 0.8% agar (w/v) (Bacto) and 3% sucrose (w/v) (Sigma) was used. The following growth regulators (0.1, 0.5, 1.0 or 1.5 mg. L⁻¹) were incorporated in the callus induction medium separately: 2,4 dichlorophenoxy acetic acid (2,4-D), a-naphthalene acetic acid (NAA) and indole acetic acid (IAA), benzyl adenine (BA) and kinetin (Kn). The pH of the medium was adjusted to 5.8 before autoclaving at 1.06 kg cm⁻² (121 °C) for 15 min. The cultures were maintained at 22 °C under 16 h photoperiod with the light intensity of 40 μmol m⁻² s⁻¹. Control cultures were initiated on MS medium without growth regulators.

All cultures were incubated for four weeks and each treatment factor consisted of 20 replicates and the experiment was repeated three times. The frequency of callus induction was calculated as the percentage of hypocotyls producing callus. To set numerical values that represented both qualitative and quantitative growth, scale rating from 0 to 9 was developed. The scale was defined as: 0- no tissue growth, 1- callus arising from one explant end, 2- callus arising from both explant ends, 3- callus arising from both explant ends and double the original explant size 4- callus arising from both explant ends and triple the original explant size 5- callus arising from both explant ends and four times the original explant size 6- callus arising from both explant ends and five times the original explant size 7- callus arising from both explant ends and six times the original explant size 8- callus arising from both explant ends and seven times the original explant size. 9- callus arising from both explants ends and eight times the original explant size.

Results and Discussion

The employment of biotechnology in plant improvement is dependent on callus induction and subsequent plant regeneration [35]. The success in callus induction is affected predominantly by the type of explant material and the in vitro culture conditions [36]. Callus regeneration is advantageous over direct regeneration for genetic transformation, since effective selection of cells having the transgene can be achieved [37].

Callusing ability of hypocotyl explants derived from 6-day-old in vitro seedlings of cv. Sultan was evaluated on MS medium supplemented with individual treatments of different auxins (2,4-D, IAA and NAA) or cytokinins (BA and Kn) (Table 1). Data were analyzed after four weeks of culture and the analysis showed that callus induction frequency, callus growth rate and nature of callus were affected by the type and concentration of the plant growth regulators.

Callusing occurred at the cut ends of the hypocotyls after five days of culture initiation. Callus induction frequency and callus growth index varied between 0-100% and 0-7.54, respectively. Generally, these parameters were higher when incorporating auxin in the medium compared to cytokinins. According to Dixon and Gonzales [38], inclusion of an auxin will be necessary for callus growth and somewhat higher auxin concentration may be required for callus initiation.

The maximum callus induction frequency was observed on MS medium fortified with 2,4-D (1.0 and 1.5 mg/l) or NAA. The auxin IAA gave a considerable level of callus induction, where 1.5 mg/l resulted in 82.8% of the culture showing callusing. NAA at 1.0 mg/l showed the best callus growth index (7.54).

Several types of calli were distinguishable based on the physical appearance. Callus induced on MS medium containing NAA or IAA (0.1-1.0 mg/l) was friable and yellow in color. However, 2,4-D resulted in brown and compacted callus.

Besides promoting slow callus growth, incorporating cytokinins (BA or Kn) in the induction media resulted in low-quality callus. When hypocotyl explants were cultured on MS media containing BA, the resulting callus were compacted and brown in color. Moreover, in case of using Kn,
the culture produced compact callus with green color. Previous studies showed that the nature of cucumber callus is largely dependent on the genotype, explant source and the plant growth regulators used. Selvaraj et al. [22] obtained nodular, greenish compact and organogenic callus in the presence of 2,4-D and BA for hypocotyls explants of cucumber cv. Poinsett 76.

Development of an efficient callus culture and plant regeneration protocol for cucumber is the first step towards the application of genetic engineering to facilitate cucumber breeding strategies. Genetic transformation of this species is desirable for the development of new varieties that are resistant to biotic and abiotic stresses [41]. In this report, we have developed a callus induction protocol for cucumber cv. Sultan. This protocol will promote the application of tissue culture technology to facilitate the genetic transformation of this species.

Table 1: Effect of different growth regulators on callus induction from hypocotyls explants of cucumber (Cucumis sativus L.) after 4 weeks of culture on MS media.

<table>
<thead>
<tr>
<th>Plant growth regulator</th>
<th>Callus induction frequency (%)</th>
<th>Callus growth index (Mean ± SE)</th>
<th>Nature of callus</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>0.00</td>
<td>0.00±0.0</td>
<td></td>
</tr>
<tr>
<td>2,4-D</td>
<td>0.5</td>
<td>6.07±0.2</td>
<td>YC</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>6.33±0.2</td>
<td>YC</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>5.20±0.1</td>
<td>BC</td>
</tr>
<tr>
<td>NAA</td>
<td>0.1</td>
<td>4.40±0.4</td>
<td>YF</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>4.73±0.4</td>
<td>YF</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>7.54±0.3</td>
<td>YF</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>7.48±0.1</td>
<td>YF</td>
</tr>
<tr>
<td>IAA</td>
<td>0.1</td>
<td>2.90±0.2</td>
<td>YF</td>
</tr>
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<td></td>
<td>0.5</td>
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<td>YF</td>
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<td>3.37±0.3</td>
<td>YF</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>4.80±0.3</td>
<td>YC</td>
</tr>
<tr>
<td>BA</td>
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<td>0.00±0.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0.80±0.2</td>
<td>YC</td>
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<tr>
<td></td>
<td>1.0</td>
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<td>YC</td>
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<tr>
<td></td>
<td>1.5</td>
<td>1.60±0.2</td>
<td>BC</td>
</tr>
<tr>
<td>Kn</td>
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<td>2.67±0.3</td>
<td>GC</td>
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<td>GC</td>
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<tr>
<td></td>
<td>1.5</td>
<td>0.67±0.1</td>
<td>BC</td>
</tr>
</tbody>
</table>

B: brown; G: green Y: yellow; C: compact; F: friable. Each value represents the treatment means of 20 independent replicates.

References
