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Rapid micropropagation of *Cucumis sativus var.* Dastgerdi *(Iranian cultivar)* by Node Culture Technique

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Tissue culture can be used in developing an efficient method for production of valuable and enduring indigenous cultivars. A short-term protocol for plantlet proliferation by using nodal segments was developed for *Cucumis sativus*, local cultivar from Isfahan; known as Dastgerdi as a tolerant cultivar to the root-knot nematode. Nodal explants were cultured on MS medium containing various concentrations of KIN (0, 0.5, 1 and 1.5mg.l^{-1}) in combination with IBA (0, 0.025 and 0.5mg.l^{-1}). The best response for proliferation rate (100%), mean number of nodes per plantlet (6.01) and percentage of rooted *In vitro* propagated shoots were observed on medium supplemented by KIN (1.5mg.l^{-1}) after about 3-4 weeks. The maximum length (5.43cm) of micro-shoots obtained on MS medium with KIN (1mg.l^{-l}) + IBA (0.025mg.l^{-1}).

Keywords: Cucumis sativus; IBA; KIN; native cultivar; proliferation.

ABBREVIATIONS

BAP, 6-Benzylaminopurine; IAA, Indole-3-acetic acid; IBA, indole-3-butyric acid; KIN, Kinetin; MS, Murashige and Skoog medium (1962).

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1. INTRODUCTION

Cucumber (*Cucumis sativus* L.) belongs to the Cucurbitaceae family. It is native to Asia, where it cultivated for more than 3,000 years and is among ten most important vegetables in the world [1]. Total production of this crop is about 57.559 million tons (MT) per year. Among the top five producers of cucumber, Iran is second producer with production 172,000 MT [2]. Cucumber is an excellent source of potassium and fiber with moderate Vitamins A and C, folic acid, phosphorous, and magnesium. It is known to be susceptible to biotic and abiotic stress [3]. Native cultivars are the naturally manipulating varieties. Genetically uniform modern varieties are replacing the highly diverse local cultivars and landraces in traditional agro ecosystems. Global concern about loss of valuable genetic resources prompted international action [4]. Displacing local varieties eroded genetic variability of regional crop populations. Local cultivar of cucumis (known as Dastgerdi) with good taste and long-term storage recognized as tolerant to the root-knot nematode, *Meloidogyne javanica*, a major pest of wide range of crops [5]. In agricultural soil, *M. javanica* can severely damage growing plants, including cucumber, tomato and melon in the hot weather areas of Iran [6]. Tissue culture is an important step toward clonal propagating plant materials, basic studies about transformation [5] and conservation of genetic resources. This is especially important for rare and endangered native plant species [7], as the method allows establishing cultures from a minimum amount of starting plant material with possible further multiplication [8]. Beside, transplanting system of *In vitro*-derived plantlets is a suitable-alternative strategy for more efficient use of greenhouse and outdoor space because seed germination and early growth of plants can be confined to a smaller nursery area [3]. Producing plants from axillary buds has proved to be the most generally applicable and reliable method of *In vitro* propagation. Besides, the chances of mutants arising with this method are far lower than with the direct/indirect shoot formation [9]. *In vitro* production of cucumber have been reported from nodal segments [10], shoot tip [3,11], cotyledon and hypocotyl explants, protoplast and leaf-derived callus and somatic embryogenesis and cell suspension cultures as indirect regeneration for mass production of this vegetable crop [6] but, to the best of our knowledge no information are available on clonal propagating selected cultivar. So, the present study was conducted to investigate the regeneration potential of *C. sativus*, var. Dastgerdi (Iran cultivar) under various concentrations of plant growth regulators, from nodal explants.

2. MATERIALS AND METHODS

2.1 Plant Materials

The experiment was carried out in the Tissue Culture laboratory Department of Agricultural Biotechnology Research Institute of Central region of Iran (ABRICI) in 2013. Seeds of *C. sativus,* native cultivar of Isfahan; known as Dastgerdi were collected from the Research Center for Agriculture and Natural Resources, Isfahan, Iran during 2012 and 2013 growing seasons.

2.2 Sterilization

For surface sterilization the seeds were washed five times with sterile distilled water, and were soaked in 0.5% Captan fungicide solution for about one hour. Then the seeds were disinfected with 0.1% $HgCl_2$ solution for 40 minutes, immersed in 70% ethanol for 1 minute and soaked in 5% sodium hypochlorite with 1 drop tween-20 for 20 minutes. Seeds were washed with distilled water after each step [3,6,10].

2.3 Culture Media and Procedure

Five Sterilized seeds were cultured per bottle containing about 30ml of MS basal medium and kept at 25ºC with illumination provided by cool white fluorescent tube at a fluency rate of 2700 Lux at medium level for 16 hd^{-1} for 7-10 days. Node culture technique [9 and10] was adopted for proliferation of seed-derived seedlings. When the seedlings reached to a height of 8 centimeters, they were excised to obtain nodal segments including 1 lateral bud in aseptic condition and placed on MS medium containing different concentrations of KIN (0.00, 0.50, 1.00 or 1.50$mg.l^{-1}$) in combination with 0.00, 0.025 and 0.50$mg.l^{-1}$ of the IBA. All cultures were incubated at the same conditions as mentioned above. After 2 weeks, the obtained shoots were rooted and subcultured onto the same proliferation medium. Transferring the shoots into the rooting medium was not needed because the shoots were rooted at the same time. The proliferation rate, length of the shoots, percentage of rooted micro-shoots and callus induction were recorded after 3 weeks.

2.4 Acclimatization

Four-week-old rooted micro-shoots transplanted into the plastic pots containing peat moss and perlite (3:1) and covered with polyethylene bag to maintain the humidity. The plantlets were acclimatized in a controlled greenhouse condition at 24ºC under 16/8 hour (light/dark) photoperiod and irrigated regularly.

2.5 Statistical Analysis

The experiment was conducted as a completely randomized factorial design with 8 replicates for each treatment (culture bottle) and 5 samples (explants per bottle) for each experiment unit. Data were analyzed using the SAS version 9.1 statistical computer program. When the ANOVA indicated significant treatment effects (5 or 1%) based on the F-test, the Duncan's multiple range test (p=0.01) was used to determine which treatments were significantly different from other treatments.

3. RESULTS

The result of ANOVA showed significant differences in the effect of different concentrations of KIN and IBA in proliferation rate, percentages of the rooted shoots per culture dish, mean number of the nodes and length of the shoots (p=0.01).

The proliferation rate showed significant differences among treatments (df 11, MS 8221.43, error 285.95, p=0.01). Based on Table 1, the highest percentage of proliferation rate (100%) was observed at (1.5$mg.l^{-1}$) KIN (Fig. 1. a, b). It is not significantly different with Kin (1.5$mg.l^{-1}$) + IBA (0.025$mg.l^{-1}$) or Kin (1.0$mg.l^{-1}$). Treatment of IBA (0.5 $mg.l^{-1}$) + KIN (0) had minimum effect on plant regeneration of nodal explants. Significant differences among treatments were observed on number of nodes (df 11, MS 2.50, error 0.97, p=0.01). Although the plantlets grown on MS medium containing KIN (1.5$mg.l^{-1}$) had the best value (6.01) of the mean number of nodes, but is not significantly different from (1$mg.l^{-1}$)KIN (Table, 1). The length of derived micro-shoots had significant differences among tested treatments (df 11, MS 12.22, error 0.47, p=0.01). According to Table 1, the maximum length of regenerated shoots (5.43 cm) was reached in culture medium supplemented with KIN (1$mg.l^{-1}$) + IBA (0.025 $mg.l^{-1}$), followed by treatment of KIN (1.5$mg.l^{-1}$) + IBA (0.025$mg.l^{-1}$). The percentage of rooted plant significantly affected by applied treatments (df 11, MS 1591.93, error 605.66,

p=0.01). All micro-shoots were successfully rooted in the same regeneration medium and so it was not needed to transfer the explants into the rhizogenesis medium (Fig. 1.c). Except two treatments of KIN (0 and 0.5mg.l^{-1}) + IBA (0.025mg.l^{-1}), none of the other treatments did not lead to callus induction (Table 1).

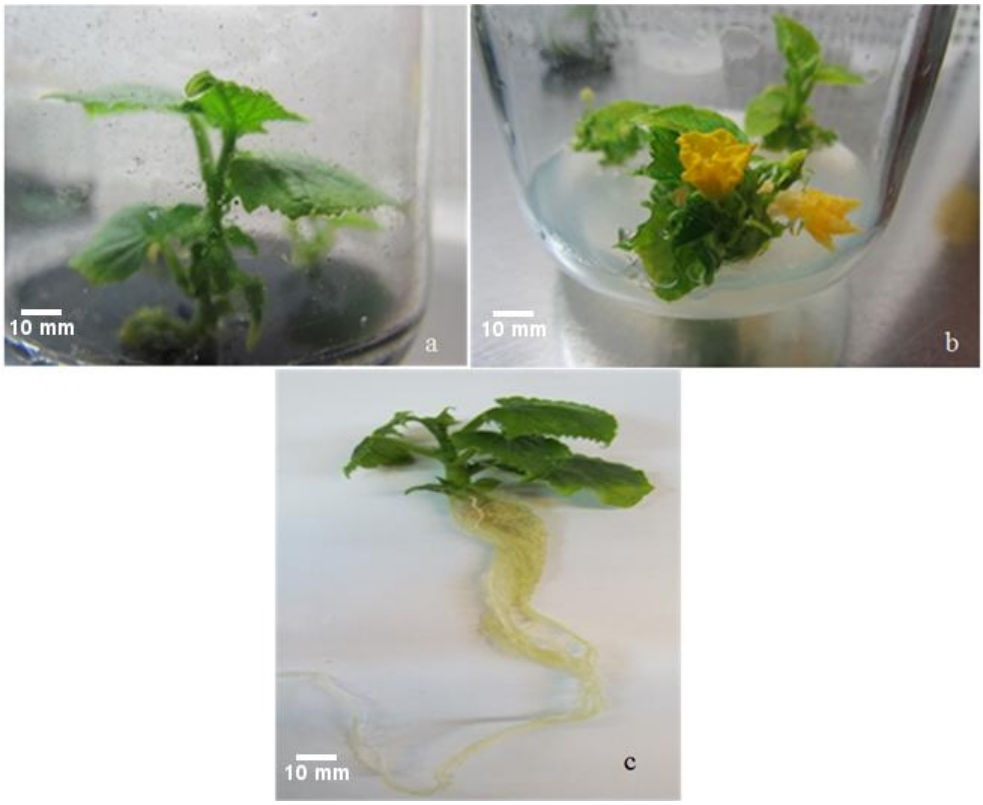

Fig. 1. *In vitro* plantlet derived from node culture after 14 days (a), 25-30 days (b) and rooted plantlets after 2 months (c)

4. DISCUSSION

The aim of this study was to study the responses of nodal explants of *Cucumis sativus* var. Dastgerdi (Iran cultivar) to culture media containing KIN and IBA. Cytokinin and auxin are the most common plant growth regulators used in *In vitro* culture of plant tissues [12]. Tissue sensitivity [13], concentration of plant growth regulators and auxin-cytokinin ratio all appear to modulate growth regulator control in cell division and plant regeneration [14]. In this study, using 1 and 1.5mg.l^{-1} of cytokinin (KIN) alone or in combination with low concentration of IBA (0.025mg.l^{-l}) showed the best proliferation rate of nodal explants. The results proved that cytokinin has a key role in shoot induction. Our results are in agreement with previous studies; reported an optimum shoot regeneration and the length of the shoots from nodal explants of *C. sativus* on MS medium containing 1.0µM BA as cytokinin and (200mg.l^{-l}) casein hydrolysate [10]. Multiple shoot induction from the shoot tip explants of *C. sativus* L. was done by Vasudevan et al*.,* 2001. A maximum number of shoots was obtained from adventitious buds after 4 weeks of culture on the medium supplemented with 1.0 mg.l^{-1} BAP or KIN [3]. Nodal cuttings of fluted pumpkin (family Cucurbitaceae) were cultured on MS

medium supplemented with combination of BA (0, 0.5, 1 and 2mg.l^{-1}) and KIN (0, 0.5 and 1mg.l^{-1}) and observed that BA and KIN at 0.5 and 1.0mg.l^{-1} respectively gave the highest number of shoots with the least callus formation [15]. The best responses for shoot multiplication on the nodal explants of *Ecballium elaterium* (L.) A. Rich. (Cucurbitaceae) were with NAA/BAP combination, followed by KIN alone and concluded that BAP responded synergistically with auxins unlike KIN [16]. According to our results and previous study, KIN alone has the ability of shoot multiplication and perhaps it could be used rather than (BAP + auxin) combination. Proliferation rate of shoot tip explants and length of derived shoots were best at 0.4µM 6-benzyladenine (BA) and the proliferated shoots developed the roots in each treatment with different frequencies in *C. sativus* [11]. The cotyledon and hypocotyl explants of *C. sativus* were cultured on MS medium fortified with different concentration (1.0-5.0mg.l^{-1}) of cytokinin BAP/KIN as individuals and in combination with IAA (0.5mg.l^{-1}) and the maximum cultures responded and number of shoots were observed in BAP (3 mg.l^{-1}) + IAA (0.5mg.l^{-1}) and KIN (3mg.l^{-1}) + IAA (0.5mg.l^{-1}) respectively [6]. The best plant growth regulator for shoot multiplication of node explants of two species of Cucurbitaceae family; pumpkin was BAP (2mg.l^{-}), and ash-gourd BAP was 1.5mg.l^{-1} [17]. Multiple shoot proliferation and rooted plantlets derived from shoot tips and stem nodes of *Cucurbita foctidissima* in MS supplemented with 1.0mg.l^{-1} BAP + 0.1mg.l^{-1} NAA [18]. Lack of the cytokinines in culture medium was associated with a decrease in the regeneration value and shoot length. These findings are in agreement to the previous research which showed cytokinines are able to induce the explants to produce the shooting responses [19]. The number of nodes bearing active buds has an important role in yield of the fruits in cucumber.

Table 1. Comparison of the effect of plant growth regulators on proliferation rate, rooted shoots per culture dish, percentage of callus induction and mean number of nodes and length of the shoots, by using Duncan's multiple range test

Treatments (mg.l^{-1})		Proliferation rate (%)	Rooted plants (%)	Number of nodes	Length of shoot	Callus induction (%)
KIN	IBA					
0.00	0.00	14.62^{e}	90.00^{abc}	4.80^{bc}	1.83^{ef}	0.00^{b}
0.00	0.025	13.20^{e}	83.25^{abcde}	4.80^{bc}	1.48^{f}	3.75^{a}
0.00	0.50	5.00^{e}	87.50^{abcd}	4.59^{bc}	1.42^{f}	0.00^{b}
0.50	0.00	71.25^{cd}	55.38^{e}	4.80^{bc}	2.70^{c}	0.00^{b}
0.50	0.025	66.25^{d}	62.25^{cde}	4.48^{bc}	2.14^{de}	2.50^{ab}
0.50	0.50	65.75^{d}	65.75^{bcde}	4.27^{bc}	1.96^{ef}	0.00^{b}
1.00	0.00	87.50^{ab}	92.50^{ab}	4.94^{ab}	2.25^{de}	0.00^{b}
1.00	0.025	83.62^{bc}	74^{abcde}	4.84^{bc}	5.43^{a}	0.00^{b}
1.00	0.50	72.50^{cd}	74.29^{abcde}	3.73^{c}	1.96^{ef}	0.00^{b}
1.50	0.00	100.00^{a}	97.50^{a}	6.01^{a}	2.90^{c}	0.00^{b}
1.50	0.025	97.75^{ab}	61.30^{de}	4.86^{bc}	4.20^{b}	0.00^{b}
1.50	0.50	67.62^{d}	89.00^{abcd}	3.99^{bc}	1.78^{e}	0.00^{b}

Within the column followed by a similar letter are not statistically significantly different (p=0.01)

In our study, the average number of nodes was raised by adding the KIN (1.5 or 1mg.l^{-1}) to the culture medium. The best medium for producing multiple buds on Balsam pear (*Momordica charantia L.)* was also Murashige and Skoog (MS) medium supplemented with 2.5mg.l^{-1} of 6-BA and low concentration of IBA (0.1mg.l^{-1}). The multiple buds were not only large but also vigorous when explants of *M. charantia* were cultured on MS medium supplemented with 2.5mg.l^{-1} 6-BA and 0.1mg.l^{-1} IBA [20]. These results agree with that previous report, which found cytokinins are usually known to promote forming buds in many *in vitro* cultured organs [21].

5. CONCLUSIONS

It is concluded KIN as cytokinin had an effective role on proliferation, shoots length and node formation of *In vitro* micropropagation of *C. sativus*. Since the explants were rooted in the same culture medium, so it was not needed to transfer the explants into the rhizogenesis medium. It is concluded that manipulating culture conditions using various combinations and concentrations of growth regulators and other adjuvants can provide a reproducible protocol and reduce the costs and time of mass production.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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