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Micropropagation of onion (*Allium cepa* L.)

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Abstract

Bulblet formation, dormancy of plantlets, vitrification of tissue and decreasing regeneration ability are the main factors limiting the efficiency of onion micropropagation. The aim of the study was experimental micropropagation of onion cvs. 'Lietuvos didieji', 'Stutgarten Riesen' and 'Centurion' F1. Onions after desinfection were split radially into four equal sectors and cross-section. Murashige and Skoog medium, supplemented with 1 mg l^{-1} naphthaleneacetic acid, 0.9, 4.4, 8.9, 13.1 μM concentrations of 6-benzylaminopurine (BAP), kinetin (1.1, 5.3, 10.6, 5.8 μM) and 30 g l^{-1} sucrose were used for plant micropropagation. The efficiency for onion micropropagation of the investigated cultivars and type of an explant was assessed according to the ability of explants to regenerate microshoots. The highest number of microshoots (1.8 to 2.4 microshoots per explant) were formed by 'Centurion' F1 and 'Lietuvos didieji' explants, containing stem dome plus basal plate. Experiments with growth regulators showed that the number of microshoots increased when the BAP concentration was raised from 0.9 to 4.4 μM, respectively from 1.0 to 2.1 microshoots per explant. The lower concentration of BAP had a higher efficiency while raised BAP concentration significantly decreased regeneration. The highest micropropagation frequency using kinetin (1.9 to 2.1 microshoots per explant) was obtained at a moderate (10.6 μM) concentration. The regeneration intensity (output of microshoots) was 68 % higher using kinetin in comparison with BAP.

Key words: *Allium cepa* L., explant, growth regulators, micropropagation, onion set.

Introduction

A real applied need for multiple *Allium* crops is in clones of economically important species like garlic (*Allium sativum* L.) and shallots (*Allium ascalonicum* L.) as well as leek (*Allium porrum* L.) and onion (*Allium cepa* L.). These species generally do not propagate by seeds. However, several tissues of onion are capable of a high potential for shoot regeneration (Mohamed-Yasseen et al. 1993), allowing to multiply a large number of useful accessions within a short time and using a small amount of a propagation material.

The first successful reports on micropropagation of onion have been described by several authors (Hussey 1978; Dunstan, Short 1979). Different parts of bulb, immature inflorescences and flowers have been used for vegetative micropropagation. Bulblet formation, dormancy of plantlets, vitrification of tissues and decreasing regeneration ability are the main factors limiting the efficiency of onion micropropagation.

The aim of the experiment was to determine the frequency of onion microshoot regeneration depending on type of the explant and concentration of different growth regulators.

Materials and methods

The experiment was carried out with two onion (*Allium cepa* L.) cultivars 'Lietuvos didieji', 'Stutgarten Riesen') and one hybrid ('Centurion' F1). Onions were grown under open field conditions with diameter from 5.0 to 10.0 mm. In total, 3840 explants of onions of different origin were plated on the regeneration medium with different concentrations of benzylaminopurine (BAP) and kinetin. Fourty explants of different types were used per treatment.

For desinfection, outer peels, roots and part of the stem dome were removed from bulb and ethanol (70 %) and mercuric chloride (0.1 %) solution (12 min) were used. After being rinsed three times in sterile water, the bulbs were split radially into four equal sectors and four cross-sections were made, dividing the bulbs into stem dome, basal plate without stem dome, stem dome with basal plate and therminal part of sheaths. Explants were planted in Petri dishes and placed in a growth chamber at 25 °C temperature, 16 h photoperiod and illumination of 30 μmol m^{-2} s^{-1}. At the first stage explants were planted onto Murashige and Skoog (1962) medium, supplemented with 1 mg l^{-1} naphthaleneacetic acid (NAA) (in all experimental variants), 0.9 to 13.1 μM BAP, 1.1 to 15.8 μM kinetin and 30 g l^{-1} sucrose. After regeneration, explants and shoots were scored and multiplied on a hormone free medium.

Results

Influence of growth regulators

The first micro shoot buds, visual with the naked eye appeared 18 to 32 days after explants were plated on the regeneration medium, respectively after 25 to 45 days, representing the first easily countable micro shoots.

Experiments with growth regulators showed that the number of microshoots increased (from 1.0 to 2.1 microshoots per explant) when the BAP concentration was raised from 0.9 to 4.4 μM. The lower BAP concentration had a higher efficiency while increasing the BAP amount insignificantly decreased regeneration. The highest micropropagation frequency, 1.9 to 2.1 microshoots per explant, was observed using the medium (10.6 μM) kinetin concentration. The output of micro shoots was 68 % higher using kinetin in comparison with BAP.

A similar increase of micro shoot regeneration has been shown by other researchers (Kahane et al. 1992). The investigated varieties differed in onion microshoot regeneration in response to growth regulators. A higher yield of shoots was obtained for explants of the cv. 'Lietuvos didieji' (mean 1 microshoot per explant) using BA, and a mean 1.5 microshoots per explant using kinetin.

Effect of explant type

Explants of the cvs. 'Centurion' F1 and 'Lietuvos didieji' had the highest frequency of regeneration, respectively 1.8 and 2.4 micro shoots per one explant. The highest number of microshoots (1.8 to 24 microshoots per explant, Table 1) were formed by 'Centurion' F1 and 'Lietuvos didieji' explants containing stem dome plus basal plate.

Morphogenesis almost did not occur from explants that consisted of the terminal part of sheaths (0.01 shoots per explant), and these explants did not produce any axillary or

Table 1. Onion (*Allium cepa*) microshoot regeneration (number of microshoots per explant ± SD) in relation to the concentration of BAP and kinetin and the type of the explant. In total 3840 explants (40 explant per treatment) were used in investigation. Data on the effect of the explant type is presented as a pooled results from all of the used concentrations of cytokinins

Variant	'Lietuvos didieji'	'Stutgarten Riesen'	'Centurion' F1
BAP 0.9 μM	0.9 ± 0.2	0.5 ± 0.1	0.7 ± 0.2
BAP 4.4 μM	1.6 ± 0.2	0.9 ±0.1	1.0 ± 0.2
BAP 8.9 μM	0.7 ± 0.1	0.5 ± 0.1	1.0 ± 0.1
BAP 13.3 μM	0.3 ± 0.1	0.8 ± 0.1	0.6 ± 0.1
Kinetin 1.1 μM	1.1 ± 0.1	0.6 ± 0.1	1.0 ± 0.1
Kinetin 5.3 μM	1.3 ± 0.1	1.0 ± 0.1	1.6 ± 0.2
Kinetin 10.6 μM	2.1 ± 0.2	1.5 ± 0.2	1.9 ± 0.1
Kinetin 15.8 μM	1.1 ± 0.1	1.2 ± 0.4	0.9 ± 0.1
Stem dome	0.1 ± 0.0	0.5 ± 0.0	1.0 ± 0.1
Stem dome plus basal plate	2.4 ± 0.2	1.4 ± 0.2	1.8 ± 0.2
Basal plate without stem dome	2.3 ± 0.2	1.4 ± 0.1	1.7 ± 0.2
Terminal part of sheaths	0.01	0.01	0.01

adventitious buds. However, they elongated and grew as single plants and some of them had intensive rhizogenesis.

Discussion

Our experiments assessed the character of onion reproduction, depending on the method of vegetative micro propagation *in vitro*. The regeneration ability of onion micro shoots depended on the concentration of cytokinin in culture medium and the type of explant. Almost no buds were observed on explants containing the terminal part of sheaths. Similarly Dunstan and Short (1979) reported that shoots were not produced from onion explants that did not include a part of apical dome. This might be related to the degree of cell differentiation in tissues of the abaxial sheath base.

Hussey (1978) suggested that axillary buds of onion could be stimulated by rupture of apical dome plus induction by cytokinin. We determined the optimal concentration of growth regulators for microshoot regeneration of onion. BAP had a higher efficiency to stimulate proliferation at the lowest concentration (< 4.4 μM). Kinetin also induced microshoot regeneration and was more effective in comparison with BAP.

References

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