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Gynogenesis pecularities of *Allium* L. vegetables grown in Lithuania

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Gynogenesis pecularities of seven species, ten varieties and different individuals of the genus *Allium* L. were investigated in 2002–2004. Flower buds were taken and sterilized, isolated on B5 medium and supplemented with 2 mg · l^{-1} 2,4-D and BA and 100 g · l^{-1} sucrose. After 30 days, the explants were transferred onto MS medium supplemented with 1 mg · l^{-1} NAA, 2 mg · l^{-1} 2iP and 100 g · l^{-1} sucrose, where gynogenic embryos as well as adventitious shoots formed. The highest number (3.2% and 4.1%) of gynogenic embryos was obtained from edible onion and Japenese leek. The gynogenesis frequency in edible onion varieties was on the average four times and the gemmagenesis eight times higher as compared with hybrids. Organogenesis frequency was different in separate plants of the edible onion variety 'Lietuvos didieji'.

Key words: adventitious shoots, *Allium* L., genotype, gynogenesis, species, variety

INTRODUCTION

Gynogenesis is one of the methods for obtaining haploids. This method is used for plant species for which androgenesis is not effective to produce haploids. Edible onion belongs to the plant group mentioned above. The first edible onion haploid was obtained in 1988 in an isolated ovule culture [1], but the frequency of embryo formation was low and the ovule culture required a lot of time for isolation. Later the development of female gametophytes was induced in an unfertilized ovary and flower culture. It has been shown that plant genotype and origin influence the frequency of gynogenesis [2, 3]. The frequency of short-day edible onion varieties reached 8%, while for long-day edible onions it was 0.4–1.7%. The different effect of gynogenesis is typical even of pure lines. Javornik et al. in 1998 compared there homozygotic lines of edible onion obtained from one genotype and determined significant differences in the production of gynogenic embryos dependent on climatic conditions [4]. The gynogenetic investigation of the genus *Allium* L. is very fragmentary.

The aim of the work was to investigate gynogenesis pecularities of different species of *Allium* L. vegetables varieties and individuals grown in Lithuania.

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MATERIALS AND METHODS

The investigation was carried out with various *Allium* L. species collected in different Lithuanian regions: chives (Alytus distr.), Japanese leek (Kaunas distr.), angle onion (Klaipëda distr.), broad-leaved garlic (Varëna distr.), vegetative onion (Skuodas distr.), as well as leek (Netherlands), edible onion varieties: 'Lietuvos didieji' (Lithuania), 'Stutgarten Riesen' (Netherlands), 'Zytawska' (Poland), 'Red Baron' (Netherlands), 'Wolska' (Poland), 'Kristine' (Poland), and 'Hyton'F_1, 'Spirit'F_1, 'Renate'F_1, 'Summit'F_1 (Netherlands).

In total, 25697 unfertilised flower buds were investigated from 132 donor plants. The donor plant were grown under open field conditions and in greenhouses of a phytotron complex at +18° ± 5 °C and 14–16 h photoperiod (additionally illuminated with a SON-T Agro-400 lamp). Umbels were taken just before the unfolding of the first flower. The collected flowers were sterilised with calcium hypochlorite (10%) solution. Flowers were rinsed three times in sterile water and planted on B5 medium [5] supplemented with 2 mg·l^{-1} 2,4-D (2,4-dichlorophenoxyacetic acid), BA (6-benzylaminopurine) and 100 gl^{-1} sucrose. On average, 50–60 explants were planted on 100 × 10 mm Petri dishes and placed in a growth chamber at a temperature of +23 °C under a 16 h photoperiod and 40 μmol^{m-2} $^{s-1}$ illumination.

After 30 days the flowers were transferred onto MS medium, [6] supplemented with 1 mg · l^{-1} NAA (naphthaleneacetic acid), 2 mg · l^{-1} 2iP (dimethylallylaminopurine), 100 g · l^{-1} sucrose.

The formation frequency (%) of explant structures, gynogenic embryos and adventitious shoots was determined.

Chromosome number was determined with a Partec ploidy analyzer and by cytological evaluation of root tip cells using the carmine staining method [7]. The data were analysed by dispersal analysis methods and grouped by the Duncan test [8].

RESULTS

Unfertilized flower buds (n = 2357) were taken from 27 donor plants were planted on B5 medium for gynogenetic efficiency estimation of different *Allium* L. species. The size of explants increased, their colour changed to creamy, light green (leek) and intensive green (chives) after 25–42 days of cultivation. Visible gynogenic embryos and adventitious shoots were formed after 37–80 days. Different organogenetic abilities were typical of the study species. The highest frequencies (3.2% and 4.1%) of gynogenic embryos were determined in edible onion and Japenese leek; the frequency of adventitious shoots was 2.5% and 2.7% in leek and chive explants, respectively (Table 1).

Edible onion varieties and hybrids started to form structures later, after 80–167 days of explant cultivation. Heterotic hybrids according to the ability to form gynogenic embryos and adventitious shoots dispersed less (0.1–4.4%) than varieties (0.8–15.0%). The local variety 'Lietuvos didieji' showed the highest ability of organogenesis. The highest output of gynogenic embryos (2.3%) was obtained from the variety 'Lietuvos didieji' (Table 2). Explants of this variety were most productive according to the production of adventitious shoots, the frequency of gemmagenesis reaching 12.7%. The gynogenesis frequency of edible onion varieties was on average four times and the gemagenesis eight times higher in comparison with hybrids.

Table 1. **Organogenesis frequency in Allium L species**

Species	Number of explants	Explant formation frequency (%*)	
		gynogenic embryos	adventitious shoots
Chives (*Allium schoenoprasum* L.)	475	0 c	2.7 a
Japanese leek (*Allium fistulusum* L.)	328	4.1 a	0 c
Angle onion (*Allium angulosum* L.)	280	0 c	1.5 b
Broad-leaved garlic (*Allium ursinum* L.)	256	0 c	1.2 b
Leek (*Allium porum* L.)	318	1.8 b	2.5 a
Vegetative onion (*Allium cepa* L. *var. agregatum*)	295	0 c	0 c
Edible onion (*Allium cepa* L.)	405	3.2 a	1.8 ab

Mean values are significantly different at $p \leq 0.05$ (Duncan's multiple range test). Values followed by the same letter do not differ significantly.

Table 2. **Organogenesis frequency of different edible onion varieties**

Variety / hybrid	Number of explants	Frequency of explant formation (%*)	
		gynogenic embryos	adventitious shoots
'Lietuvos didieji'	5410	2.3 a	12.7 a
'Kristine'	2212	0.1 d	0.7 d
'Štutgarten Riesen'	1418	0 d	0.5 d
'Red Baron'	1112	0.2 cd	3.1 b
'Zytawska'	655	0 d	1.5 c
'Wolska'	582	0.1 cd	0.7 d
'Renate' F_1	712	1.3 b	3.1 b
'Hyton' F_1	875	0.3 c	3.1 b
'Spirit' F_1	1404	0 d	0.1 e
'Summit' F_1.	954	0.1 d	3.8 b
Total for varieties	11389	1.1 a	6.7 a
Total for hybrids	3945	0.3 b	2.2 b
Total	15334	1.4	8.9

*Means are significantly different at $p = 0.05$ (Duncan's multiple range test). Values followed by the same letter do not differ significantly.

Table 3. **Ploidy level in isolated flower culture of *Allium* L. regenerated plants**

Species	Number of plants from gynogenic embryos			Number of plants from adventitious shoots		
	haploids	diploids	mixoploids	haploids	diploids	mixoploids
Edible onion	54	13	7	9	390	23
Chives	9	0	1	0	11	1
Japanese leek	0	0	0	0	2	0
Angle onion	0	0	0	0	4	0
Broad-leaved garlic	5	0	1	0	2	1
Leek	0	0	0	1	14	1

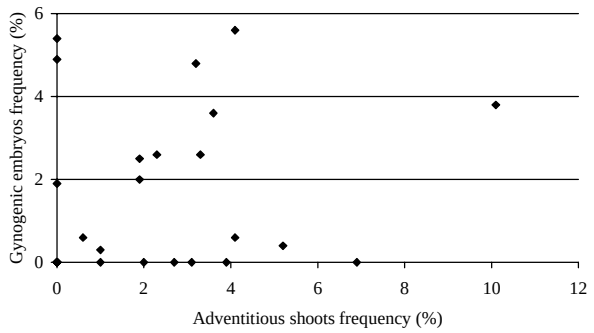

Figure. Scatter plot of edible onion 'Lietuvos didieji' genotypes based on organogenesis values

The obtained results encouraged to investigate the pecularities among the individuals in a population. In total, 8006 unfertilized flower buds isolated from 30 different plants of the variety 'Lietuvos didieji' were planted on B5 medium. Two different groups of plants can be distinguished according to the frequency of organogenesis. The frequency of gynogenic embryos was typically 0 to 1% in the first group (Figure). The genotypes of this group formed adventitious shoots with a 6.9% frequency. A significantly higher frequency (up to 6.5%) of gynogenic embryos was obtained in the the second group.

The majority (90%) of plants that regenerated during gemmagenesis had a diploid chromosome number, and 87% of regenerants formed during gynogenesis were haploid (Table 3).

DISCUSSION

One of the most important factors influencing the efficiency of gynogenesis is plant donor genotype [9–11]. Various species differ in this aspect. Gynogenesis pecularities in edible onion from the genus *Allium* are investigated best. Gynogenesis investigations in other *Allium* L. species was either not studied or data are very fragmentary. Four species of seven showed a positive gynogenetic response. Gynogenesis frequency of different sugar beet varieties varied from 0 to 16%, of leek from 0 to 2% and in red beet reached 11% [11–13]. The obtained results showed that gynogenesis frequency of the edible onion varieties studied was four times higher than in hybrids. It is possible that a higher diversity is typical of varieties (populations) created on a wide genetic basis). It was demonstrated that the variety population 'Lietuvos didieji' according to organogenetic response in isolated flower culture can be divided into several groups, implying that different plants with a higher regenerant formation frequency can be selected in a population.

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LIETUVOJE AUGINAMØ *ALLIUM* L. GENTIES DARÞO AUGALØ GINOGENEZËS YPATUMAI

Santrauka

2002–2004 m. ištirta *Allium* L. genties septyniø rûðiø, deðimties veisliø populiacijos bei atskirø individø ginogene-

zës ypatumai. Augalai donorai auginti lauke ir fitotroninio komplekso ðiltnamiuose, taip pat augalø auginimo kamerose. Nuskinti ir sterilizuoti þiedø butonai buvo izoliuoti ant B5 maitinamosios terpës, papildytos 2 mg/l 2,4-D ir 2 mg/l BAP. Praëjus 30 parø, eksplantai augalø regeneracijai indukuoti buvo perkelti ant MS maitinamosios terpës, papildytos 1 mg/l NAR, 2 mg/l iP ir 100 g/l sacharoze, ant kurios ðalia ginogeniniø embrionø taip pat vystësi ir adventyviniai ûgliai. Daugiausiai – 3,2% ir 4,1% ginogeniniø embrionø nustatyta auginant valgomøjø svogûnø ir tuðèialaiðkiø èesnakø eksplantus. Valgomøjø svogûnø veisliø ginogeniniø embrionø formavimosi daþnis vidutiniðkai 4 kartus, o adventyviniø ûgliø – 8 kartus buvo didesnis nei hibridø. Ðiuos skirtumus lëmë nevienodas heterozigotiðkumo laipsnis ir populiacijø genetinë ávairovë. Tarp veislës ‘Lietuvos didieji’ augalø nustatyti organogenezës daþnio skirtumai, kurie priklausë nuo ðiø augalø genetinës prigimties.