Improvement of in vitro gynogenesis induction in onion (Allium cepa L.) using polyamines

Liliana E. Martínez a,*, Cecilia B. Agüero a, María E. López a, Claudio R. Galmarini b

a Cátedra de Fisiología Vegetal, Facultad de Ciencias Agrarias, CC No. 7, 5505, Chacras de Coria, Mendoza, Argentina
b Estación Experimental INTA La Consulta, CC 8, 5567, La Consulta, Mendoza, Argentina

Received 20 September 1999; received in revised form 15 March 2000; accepted 16 March 2000

Abstract

The effects of polyamines on gynogenetic embryogenesis and regeneration of plantlets in onion were studied. Whole flowers from two onion genotypes, ‘Valcatorce INTA’ cultivar and ‘Torrentina’ population, were used as initial explants. Embryo induction was greatest with a combined treatment of 2 mM putrescine and 0.1 mM spermidine. Addition of putrescine alone, with a few exceptions, did not have any significant effect on either embryo induction or haploid plantlet production for both onion genotypes. ‘Torrentina’ showed a higher embryo generation capacity (9.5%) than ‘Valcatorce INTA’ (2.8%). Fast regeneration of embryos was achieved (from 60 to 90 days) as compared to a previously reported time of 46–152 days. The use of spermidine (0.1 mM) after 15 days of culture promoted further embryo maturation and plantlet formation. ‘Torrentina’ produced more haploid plants (1.90%). This is the first report of successful use of polyamines for induction of gynogenic embryos and regeneration of onion plantlets. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Allium cepa L.; Onion; Haploids; In vitro culture; Gynogenesis; Polyamines; Breeding

1. Introduction

The onion crop is of great importance in Argentina and is one of the main exported fresh vegetables. For decades there have been well-established onion breeding and seed production programs in Argentina, developing short, intermediate and long-day cultivars. Today almost 90% of the cultivars used come from local breeding programs. The most successful cultivar is ‘Valcatorce INTA’, representing 80% of the onion area in the country: it is also cropped in Chile and Uruguay. The main onion breeding program is located at La Consulta Experimental Station (INTA). The breeding goals are early harvesting and good storage quality in long-day cultivars, and introduction of pink-root resistance and high dry matter into cultivars for the dehydration industry [1]. In the breeding program classical methods, like mass selection, pedigree, recurrent selection and hybrid production are used, together with in vitro culture techniques (e.g. ovule and ovary culture) to hasten the production of homozygous lines. At present several haploid plants have been obtained [2].

The use of haploidization methods in breeding hybrid cultivars is of particular interest in long cycle species [3], since at least 6–10 years are required to produce inbred lines [4]. Treatment of haploid plants with colchicine can produce superior homozygous diploid lines, which can be used to create hybrids with desirable features in considerably shorter time.

Abbreviations: BA, 6-benzylaminopurine; BDS, Dunstan and Short medium; B5, Gamborg medium; MS, Murashige and Skoog medium; NAA, naphtalenacetic acid.

* Corresponding author. Tel.: 54-261-4960004, ext. 2036; fax: +54-261-4960469.
E-mail address: lmartinez@fca.uncu.edu.ar (L.E. Martínez).
Different techniques have been used to obtain haploid plants: induced parthenogenesis by interspecific or intergeneric crosses, use of inactivated pollen and in vitro culture of male or female gametophytes [5].

The induction of onion haploid plants has recently been achieved in vitro via gynogenesis [2,6–15], and even in situ after crossing onion plants with irradiated pollen [16]. Gynogenic onion embryos have been obtained from ovules, ovaries and whole flowers, using various media and culture conditions. However, in spite of many efforts trying to improve the haploid induction technique, the yield of gynogenic embryos and haploid plants obtained is frequently very low [2,6–15].

Cellular and morphogenetic events during somatic embryogenesis are controlled by an array of culture conditions (medium composition and physical environment) and by genetic effects [17,18]. The genotype of donor plants has a big influence on onion haploid induction ability [4,14,15]. Inbreds and synthetics tend to have higher rates of embryo production and plant regeneration, compared to open populations where the response is generally lower [14].

Polyamines (agmatine, putrescine, spermidine, spermine, cadaverine, etc.) are normal plant growth regulators involved in all growth or developmental process in plants [19,20]. They occur in high amounts in the flowers of various plants [20–24]. An increase in polyamine biosynthesis has been shown to precede or accompany calllogenesis [24], organogenesis [25] and somatic embryogenesis [26] in a number of plant cell cultures [27–30]. Danin et al. [28] suggested that the onset of embryogenesis in celery is characterized by a high content of putrescine and cytokinin, while a decrease in putrescine synthesis and cytokinin content and an increase in spermidine and spermine content accompany further embryo development and plantlet formation. Despite several experiments in a wide range of species with these growth regulators, the role of polyamines in different species, in in vitro gynogenesis has not been elucidated, not even in onion.

The present research was designed to determine, for the first time, the effects of different polyamines in the production of embryos and haploid onion plants through in vitro gynogenesis.

2. Material and methods

2.1. Plant material

Experiments were carried out during the growth period of November, 1995 and August, 1996. The genotypes used were a long-day cultivar ‘Valcatore INTA’ and an intermediate-day population, ‘Torrentina’. Young flowers were collected from the umbels 3–5 days before anthesis, surface-sterilized with 10% sodium hypochlorite solution containing a few drops of Tween 20 for 15 min, and rinsed three times with sterile distilled water.

2.2. Media and culture conditions

Flowers were cultured on induction medium consisting of Dunstan and Short medium (BDS) macro [32], Gamborg medium (B5) microelements [33] and Murashige and Skoog medium (MS) vitamins [34], supplemented with 100 g/l sucrose (medium A). Putrescine treatments consisted of 0 mM (control P0), 0.1 mM (P1), 0.5 mM (P2), 1 mM (P3) and 2 mM (P4) concentrations. After 15 days of culture, flowers were transferred to a regeneration medium: medium A with 0.1 mM of spermidine (S1) or without spermidine (control S0).

Gynogenic embryos breaking through the ovary wall were transferred to embryo culture medium composed of macro, microelements and vitamins according to MS [34], without growth regulators and supplemented with 40 g/l sucrose and 7.5 g/l agar (medium B). The explants were grown until complete plant development was reached. After 40 days of culture on the previous medium, the regenerated plantlets were removed and cultured on micropropagation medium: medium B plus 2 mg/l naphtalenacetic acid (NAA) and 2 mg/l 6-benzyllaminopurine (BA).

All media were adjusted to pH 5.8, solidified with 0.75% agar and sterilized by autoclaving at 121°C for 20 min. Petri dishes containing 30 ml of medium and 30 flowers each were placed under a 16-h photoperiod (30 μmol/s per m2, supplied by Phillips cool-white fluorescent bulbs), at 25–20 ± 2°C.

The number and percentage of gynogenic embryos, entire plants and haploid plantlets were scored. The experimental design was a randomized plot design with ten and five replications for ‘Val-
After 30 days of culture, ovaries were two to three times their original size. Some of them broke spontaneously and showed black ovules, which looked like fully developed seeds.

Anther dehiscence was not observed, so it was assumed that in vitro pollination did not occur. These findings support the observations of Smith et al. [9].

The gynogenic embryos were easily distinguished, coming out from the ovules. They appeared 60–90 days after inoculation of the flowers on induction medium (Fig. 1). This result suggests an extremely fast regeneration of embryos, which is a desired characteristic to reduce the total time required for in vitro gynogenesis.

In some cases, direct plantlet regeneration was observed from the septal nectaries region. The ploidy level of these plantlets was diploid (2n = 16) due to their somatic origin, so they were not taken into account for further studies.

For ‘Valcatorce INTA’, the embryo yield obtained in media containing spermidine along with increasing concentration of putrescine was significantly higher than in putrescine media or polyamines-free media (control) (Table 1). ‘Torrentina’ showed similar behavior. The maximum yield (9.5%) was achieved with 2 mM putrescine and subsequent culture on 0.1 mM spermidine (Table 2). Lower putrescine concentration significantly reduced embryogenesis in this population compared to the P4-S1 medium. ‘Torrentina’ showed higher embryo generation capacity compared to the ‘Valcatorce INTA’.

This is the first report showing a positive effects of polyamines for embryo induction in onion or in other species.

Previous reports have shown a lesser percentage of gynogenic embryos from different genotypes, organs and culture media, ranging from 0.27 to 7.6% [2,4,7,8,11,13,12]. However, recently Bohanec and Jakše [15] found average embryo yields of 18.6–22.6% for line and 51.7% for individual plants, indicating that gynogenesis is strongly affected by the genotype.

Fig. 1. Gynogenic embryo breaking through the carpel.
Several studies demonstrate a role for polyamines in carrot somatic embryogenesis [27] and in celery embryo differentiation and plantlet development [28]. In the present work, the addition of putrescine to the medium during the first 15 days of culture followed by addition of 0.1 mM spermidine induced the onset of embryogenesis and increased the number of gynogenic embryos obtained.

The embryo production phase is crucial to improve the yield of regenerated plantlets, as the higher the gynogenic embryo number, the higher probability of obtaining haploid plants. There were significant differences in plantlet regeneration between the two onion populations. The mean percentage of embryos which developed into whole plantlets (haploids and diploids) in ‘Torrentina’ was 82% while in ‘Valcatorce INTA’ it was 33%. This supports the findings of Phillips and Luteyn [31], Bohanec et al. [4] and Gioffria et al. [14], who emphasized that the production of onion haploid plants was strongly affected by the population.

Spontaneous diploidization in onion occurs in the roots [6] and shoot apices [13]. In the present study, the ploidy of the regenerants was established by counting the chromosomes and gave only \( n = 8 \) (Fig. 2) for haploid plantlets. Diploid plantlets were observed, but they probably originated from diploid somatic tissue and not from spontaneous duplication in haploids.

The highest percentage of haploid plantlets obtained from ‘Valcatorce INTA’ (1.0%) was reached with the higher concentration of putrescine (2 mM) and subsequent culture on 0.1 mM spermidine (Fig. 3). ‘Torrentina’ showed 1.9 and 1.4% regeneration rates of haploid plantlets with the P4-S1 and P2-S1 media, respectively (Fig. 3).

### Table 1
Effects of putrescine (P) and spermidine (S) on the gynogenetic embryo and plantlet yields of ‘Valcatorce INTA’ cultivar

<table>
<thead>
<tr>
<th>Treatments</th>
<th>No. of flowers</th>
<th>Gynogenic embryos</th>
<th>Total regenerated haploid plantlets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%)</td>
<td>No.</td>
<td>No.</td>
</tr>
<tr>
<td>Control P0-control S0</td>
<td>301</td>
<td>3* (0.9)</td>
<td>1*</td>
</tr>
<tr>
<td>Control P0-S1</td>
<td>330</td>
<td>4ab (1.2)</td>
<td>2*</td>
</tr>
<tr>
<td>P1-control S0</td>
<td>298</td>
<td>7b (2.3)</td>
<td>3b</td>
</tr>
<tr>
<td>P1-S1</td>
<td>313</td>
<td>3b (1.6)</td>
<td>3b</td>
</tr>
<tr>
<td>P2-control S0</td>
<td>295</td>
<td>3b (1.0)</td>
<td>2*</td>
</tr>
<tr>
<td>P2-S1</td>
<td>251</td>
<td>7b (2.8)</td>
<td>1*</td>
</tr>
<tr>
<td>P3-control S0</td>
<td>290</td>
<td>2a (0.7)</td>
<td>0a</td>
</tr>
<tr>
<td>P3-S1</td>
<td>263</td>
<td>5b (1.9)</td>
<td>0a</td>
</tr>
<tr>
<td>P4-control S0</td>
<td>268</td>
<td>2a (0.7)</td>
<td>0a</td>
</tr>
<tr>
<td>P4-S1</td>
<td>305</td>
<td>6b (2.0)</td>
<td>4b</td>
</tr>
<tr>
<td>Total</td>
<td>2914</td>
<td>44 (1.5)</td>
<td>16</td>
</tr>
</tbody>
</table>

*Means within each column followed by the same letter are not significantly different at \( P \leq 0.05 \) (Tukey’s test).

### Table 2
Effects of putrescine (P) and spermidine (S) on the gynogenetic embryo and plantlet yields of ‘Torrentina’ population

<table>
<thead>
<tr>
<th>Treatments</th>
<th>No. of flowers</th>
<th>Gynogenic embryos</th>
<th>Total regenerated haploid plantlets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%)</td>
<td>No.</td>
<td>No.</td>
</tr>
<tr>
<td>Control P0-control S0</td>
<td>173</td>
<td>1b (0.6)</td>
<td>1b</td>
</tr>
<tr>
<td>Control P0-S1</td>
<td>180</td>
<td>2b (1.1)</td>
<td>2b</td>
</tr>
<tr>
<td>P2-control S0</td>
<td>100</td>
<td>0a (0)</td>
<td>0a</td>
</tr>
<tr>
<td>P2-S1</td>
<td>147</td>
<td>2b (1.4)</td>
<td>2b</td>
</tr>
<tr>
<td>P4-control S0</td>
<td>108</td>
<td>1b (0.9)</td>
<td>1b</td>
</tr>
<tr>
<td>P4-S1</td>
<td>105</td>
<td>10c (9.5)</td>
<td>9c</td>
</tr>
<tr>
<td>Total</td>
<td>813</td>
<td>16 (2.0)</td>
<td>15</td>
</tr>
</tbody>
</table>

*Means within each column followed by the same letter are not significantly different at \( P \leq 0.05 \) (Tukey’s test).
Although a combination of BA and 2,4-D has been used by numerous authors [3,4,7,11–15] to induce onion gynogenesis with success, in the present report we did not use them, because previous results working with the same genotypes yielded a smaller percentage of haploids plantlets (0.36–0.65%) in ‘Valcatorce INTA’ cultivar [2] in comparison with polyamines.

Fig. 2. Eight chromosomes in root tip cells of haploid regenerated plantlet.

Fig. 3. Effect of polyamines on the production of haploid plantlets of ‘Valcatorce INTA’ cultivar.

Fig. 4. Effect of polyamines on the production of haploid plantlets of ‘Torrentina’ population.

According to these results, the use of spermidine in the media should promote further embryo maturation and plantlet formation in onion. Moreover, the percentage of haploid plants obtained with ‘Valcatorce INTA’ and ‘Torrentina’ represents an important improvement in the gynogenesis technique used in onion, for the production of haploid and doubled haploid plants. ‘Torrentina’ appeared to be the most responsive to produce haploid plants.

The percentage of haploid plantlets obtained using polyamines allows use of this methodology in onion breeding programs; consequently large number of accessions could be used in a more efficient way. Further experiments are in progress to duplicate the chromosome number of haploid plants to hasten the production of homozygous lines for hybrid production.

Acknowledgements

This research was supported by a grant from CIUNC (Consejo de Investigaciones de la Universidad Nacional de Cuyo). The authors thank Dr K. Bradford for critical review of the manuscript.

References


