Iyyakkannu Sivanesan, Mi Young Lim, Eun Hae Jo,  
Byoung Ryong Jeong*  
Department of Horticulture, Division of Applied Life  
Science (BK21 Program), Graduate School, Gyeongsang  
National University, Jinju, Korea 660-701  
e-mail: brjeong@gnu.ac.kr

Abstract—A simple and efficient protocol was developed for  
somatic embryogenesis from corm explants of *Crocus vernus*.  
Microcorms obtained from field grown plants were  
decontaminated and divided into several parts, cultured on the  
Murashige and Skoog (MS) medium supplemented with 3%  
(w/v) sucrose, 0.8% (w/v) agar, and different concentrations  
and combinations of plant growth regulators. Somatic embryos  
were induced both light and dark conditions but culturing the  
explants two weeks in the dark followed by three weeks under  
light resulted in high frequency of embryo formation. The  
greatest percentage of embryo induction was achieved when  
the explants cultured on the MS medium with 0.5 mg L⁻¹  
thidiazuron (TDZ) and 0.1 mg L⁻³ naphthaleneacetic acid (NAA).  
Embryo maturation and germination were achieved on a MS  
medium with 2.0 mg L⁻¹ BA and 1.0 mg L⁻³ GA₃. When the  
globular embryos were transferred to the MS medium  
containing 6% (w/v) sucrose, 2.0 mg L⁻¹ BA, and 1.0 mg L⁻³  
GA₃ resulted in the highest frequency of plant regeneration  
and microcorm formation. The microcorms developed new  
shoots when they were cultured on the half-strength MS  
medium with 1.0 mg L⁻¹ GA₃.

Keywords—Crocus; microcorm; somatic embryogenesis;  
Thidiazuron

I. INTRODUCTION

The genus *Crocus* with about 85 species comprises  
important medicinal and ornamental plants. *Crocus vernus*  
(L.) Hill is a perennial herbaceous species commonly known  
as Dutch crocus that has a wide distribution in Europe, North  
Africa, and temperate Asia. It is typically propagated by  
corms. Low rate of daughter corm formation under natural  
conditions is limited availability of propagating material for  
field cultivation. Tissue culture techniques can play an  
important role in the clonal propagation and qualitative  
improvement of this important plant species. Moreover, in  
vitro propagation of plants could help in raising disease free  
healthy clones on a large scale for the horticultural industry.  
Large scale production of elite clones through  
micropropagation or somatic embryogenesis solves this  
problem. Clonal propagation through somatic embryogenesis  
has become an essential method for the improvement of most  
economically important plants [1]. Direct embryogenesis  
reduces the time required for plant propagation, which may  
be beneficial to minimize culture-induced genetic changes.  
Somatic embryogenesis offers an excellent experimental  
system to study the physiological and biochemical aspects of  
embryo development [2]. Somatic embryos are of extensive  
practical importance in large scale propagation [3], genetic  
manipulations [4], cryopreservation [5], and artificial seed  
production [6]. Although tissue culture in the *C. vernus*  
has been previously reported [7], to date there is no report on  
somatic embryogenesis and plant regeneration. The aim of  
this study was to develop a reliable and efficient in vitro  
propagation protocol for *C. vernus*.

II. MATERIALS AND METHODS

The corms were collected from greenhouse-grown plants,  
washed thoroughly in running tap water for 30 min, and then  
was washed with distilled water. The explants decontaminated  
with a 70% (v/v) ethanol for 60 sec, 2.0% sodium  
hyPOCHLORITE for 10 min, and 0.01% mercuric chloride for 15  
min. Each treatment was followed by 3-4 washes with sterile  
distilled water, cut into 0.5-1.0 cm segments and cultured on  
Murashige and Skoog (MS) medium supplemented with  
different concentrations and combinations of BA and NAA.  
Hormone-free MS medium was used as the control. Number  
of explants initiating shoot buds and average number of  
shoot buds per explant were recorded after 45 days. In each  
experiment, about 25 explants were used and the experiment  
was repeated thrice.

The medium consisted of MS basal salts and vitamins [8],  
supplemented with 3% (w/v) sucrose, and solidified with 0.8%  
(w/v) agar. The pH of the medium was adjusted to 5.7 using  
0.1 N NaOH or 0.1 N HCl before autoclaving at 121 °C for  
15 min. Gibberellic acid (GA₃) and thidiazuron (TDZ) were  
filter sterilized and added to autoclaved medium. Other plant  
growth regulators were added to basal medium prior to pH  
adjustment and sterilization. All cultures were maintained at  
25±2°C under a 16 h photoperiod with 30 µmol m⁻² s⁻¹  
irradiance provided by cool white fluorescent light (PHILIPS  
40 W tubes) unless otherwise stated.

Corm and shoot bud explants were cultured on the MS  
medium supplemented with TDZ and NAA. The explants  
were maintained for 0, 7 and 14 days at 25±2°C in darkness  
and then exposed to light of 30 µmol m⁻² s⁻¹ with a light/dark  
cycle of 16/8 h. In each experiment, about 25 explants were  
used and the experiment was repeated thrice. The frequency  
of somatic embryo formation was determined by counting  
explants forming somatic embryos from the total number of  
cultured explants after 45 days of culture. Somatic  
embryos (globular stage) were separated from the explants  
and cultured on the MS medium supplemented with sucrose.
(3% or 6%, (w/v), 2.0 mg L\(^{-1}\) BA and 1.0 mg L\(^{-1}\) GA\(_3\) for maturation and germination. Somatic embryos were sub-cultured at intervals of 3 weeks. Embryo germination percentage was calculated as (number of germinated somatic embryos/ total number of somatic embryos) × 100.

Data were statistically analyzed by analysis of variance (ANOVA) followed by Duncan multiple range test at 5% probability level. Data analysis was performed using SAS computer package (SAS Institute Inc., NC, USA).

III. RESULTS AND DISCUSSION

When corm explants of *C. vernus* were cultured on the MS basal medium, they did not induce shoot buds or callus. In contrast, when the same explants were grown on the MS medium containing PGRs they produced shoot buds. When MS medium was supplemented with different concentrations of BA, shoot buds emerged from the corm explants starting 4 weeks after culture. Plessner et al. (1990), reported that Shoot development on corm explants was promoted by cytokinins. The highest percentage of shoot formation with an average number of 2.0 shoots per explant was observed on MS medium supplemented with 2.0 mg L\(^{-1}\) BA (Table 1).

In general, a combination of cytokinin and auxin enhanced shoot bud induction. The frequency of shoot induction and number of shoots increased when BA was combined with NAA in comparison to BA alone. The greatest percentage of shoot induction (94.2) with a mean number of 4.3 shoots per explants was achieved when the medium was supplemented with 2.0 mg L\(^{-1}\) BA and 0.5 mg L\(^{-1}\) NAA (Fig 1A). Similar results were obtained with the same hormones by Bhagyalakshmi [9]. and Karaoglu et al. [10].

![Fig. 1. Direct shoot regeneration and somatic embryogenesis of Crocus vernus. A, shoot regeneration from corm explants after 45 days of culture; B and C, somatic embryo formation from shoot tip explants of *C. vernus* after 45 days of culture; D and E, somatic embryo formation from corm explants of *C. vernus*; and F, cotyledonal stage.](image)

<table>
<thead>
<tr>
<th>Conc. mg L(^{-1})</th>
<th>Shoot induction (%)</th>
<th>No. of shoots/explant</th>
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<tr>
<td>BA</td>
<td>NAA</td>
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<tr>
<td>0</td>
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</tr>
<tr>
<td>0.5</td>
<td>0</td>
<td>72.8 e</td>
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<tr>
<td>1.0</td>
<td>0</td>
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<tr>
<td>2.0</td>
<td>0</td>
<td>86.0 e</td>
</tr>
<tr>
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<td>0.5</td>
<td>91.0 b</td>
</tr>
<tr>
<td>2.0</td>
<td>0.5</td>
<td>94.2 a</td>
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Means followed by same letter (s) within a column are not significantly different (P<0.05)

The formation of somatic embryos was observed when corm and shoot bud explants were cultured on the MS medium was supplemented with TDZ and NAA (Fig. 1B-E). Of the two explants, shoot bud explants found to be the most effective for somatic embryo induction. Corm and shoot bud explants produced maximum number of somatic embryos at 0.5 mg L\(^{-1}\) TDZ and 0.1 mg L\(^{-1}\) NAA and the percentage of somatic embryo induction were 97 and 100, respectively (Table 2). Embryo maturation and germination were achieved on the MS medium with 2.0 mg L\(^{-1}\) BA and 1.0 mg L\(^{-1}\) GA\(_3\) (Fig. 2B). When the globular embryos were transferred to the MS medium containing 6% (w/v) sucrose, 2.0 mg L\(^{-1}\) BA, and 1.0 mg L\(^{-1}\) GA\(_3\) resulted in the highest frequency of plant regeneration and microcorm formation (Fig. 2A). The microcorms developed new shoots when they were cultured on the half-strength MS medium with 1.0 mg L\(^{-1}\) GA\(_3\).

<table>
<thead>
<tr>
<th>Conc. mg L(^{-1})</th>
<th>Somatic embryo induction (%)</th>
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<tr>
<td>TDZ</td>
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<td>78.2 d</td>
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<tr>
<td>0.5</td>
<td>97.0 a</td>
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<tr>
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<td>90.3 b</td>
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<tr>
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<td>88.7 c</td>
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Means followed by same letter (s) within a column are not significantly different (P<0.05)
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REFERENCES


